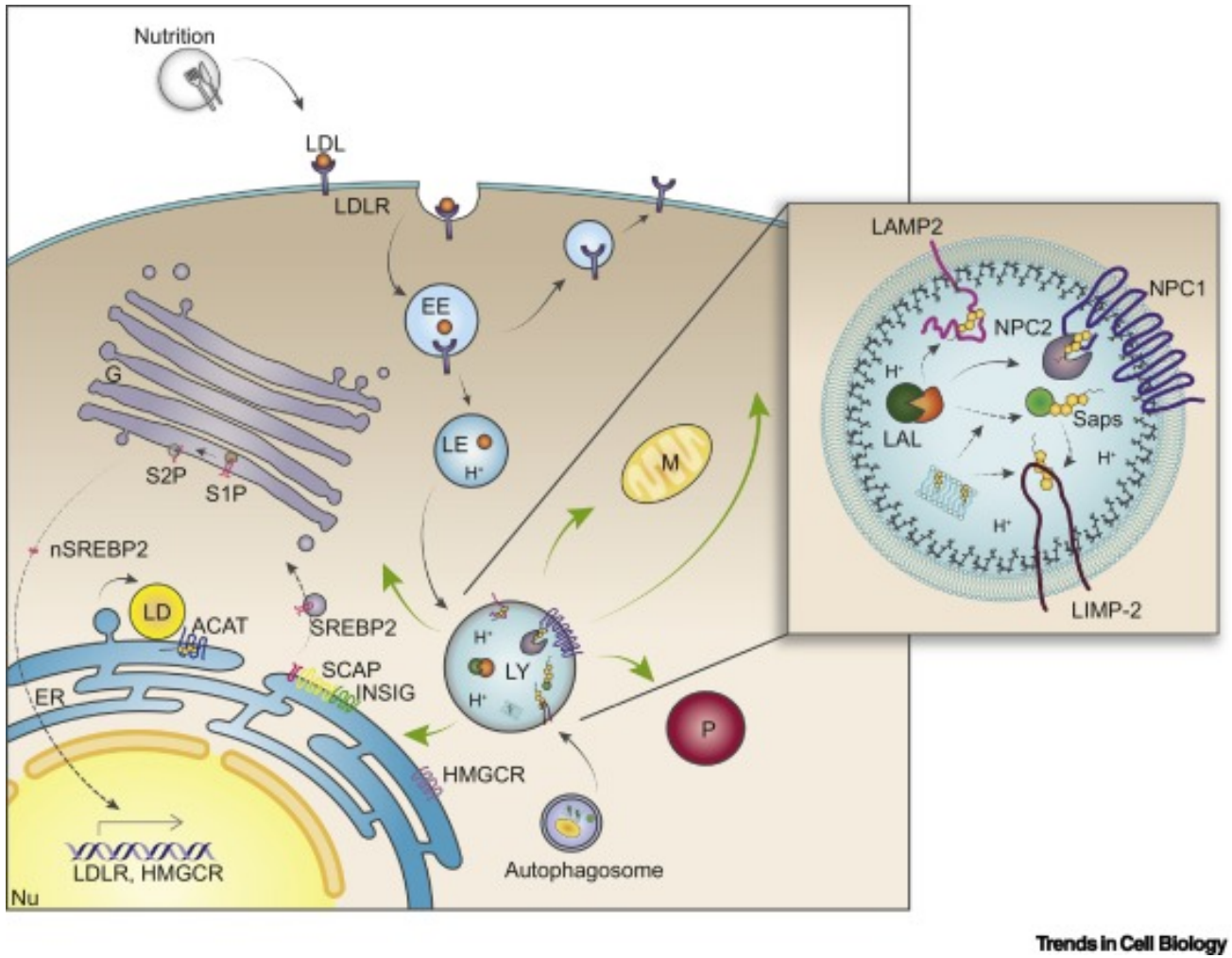
Identifying and studying cellular compartments & membranes using light-activated tools

Journal Club

12.01.2021

Alexandra Bentrup

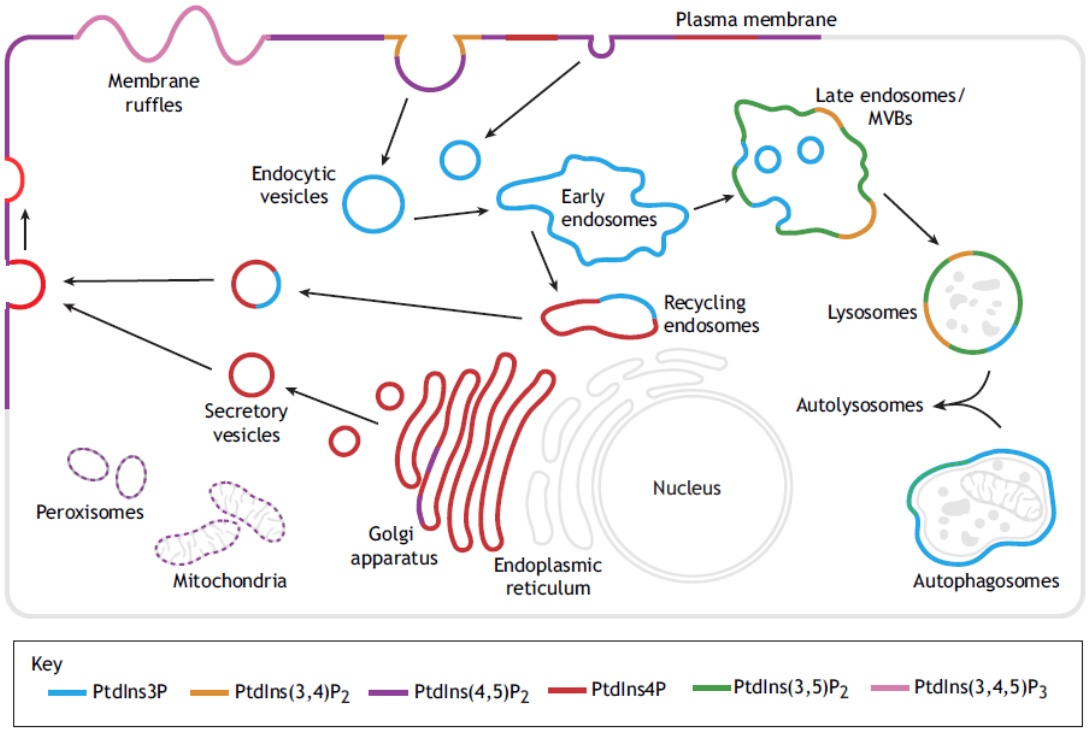
Identity of cellular compartments



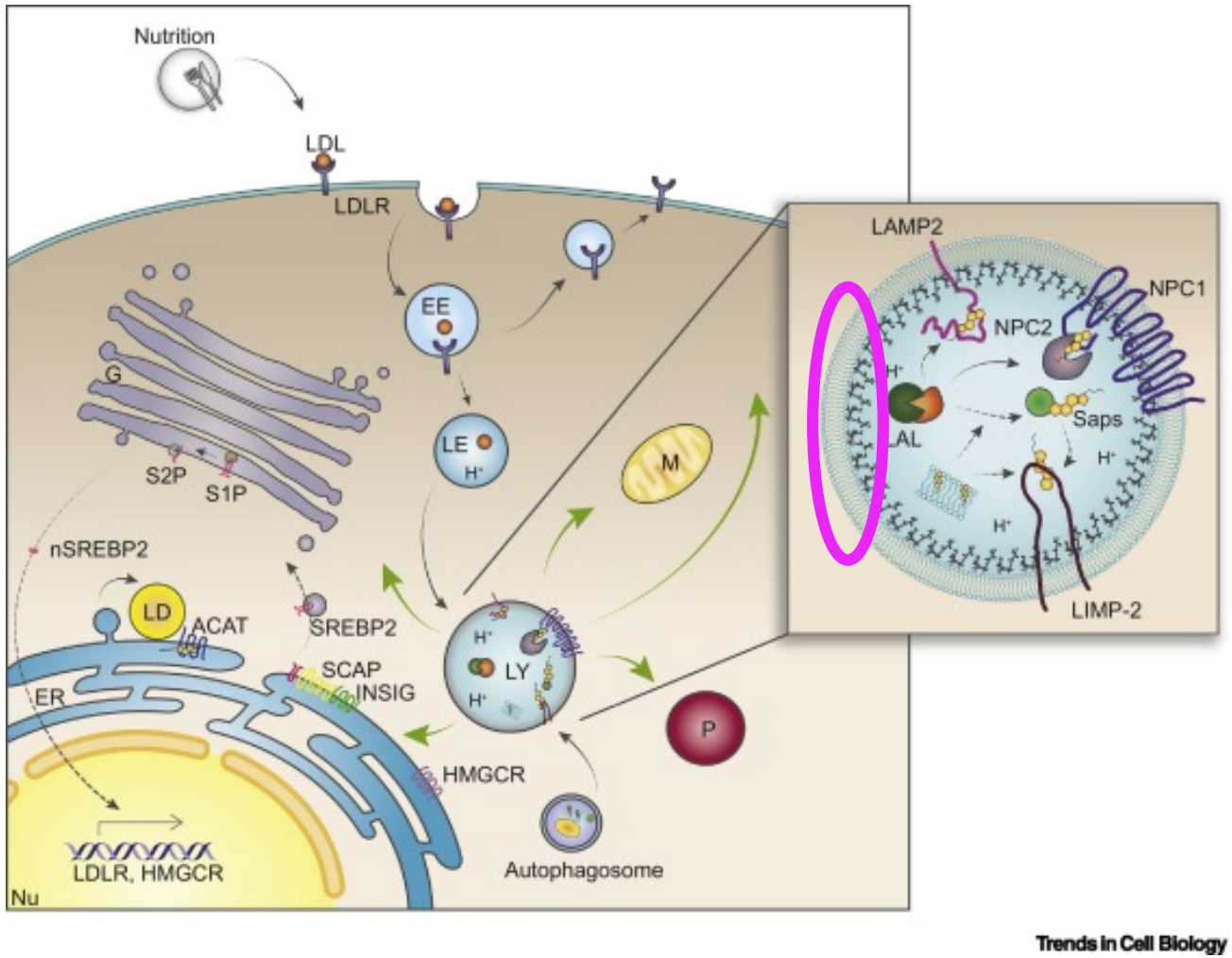
Meng, 2020, Trends in Cell Biology

Identity of cellular compartments:

- Lipid composition, e.g. phosphoinositides
  PIKfyve: PtdIns3P → PtdIns(3,5)P2



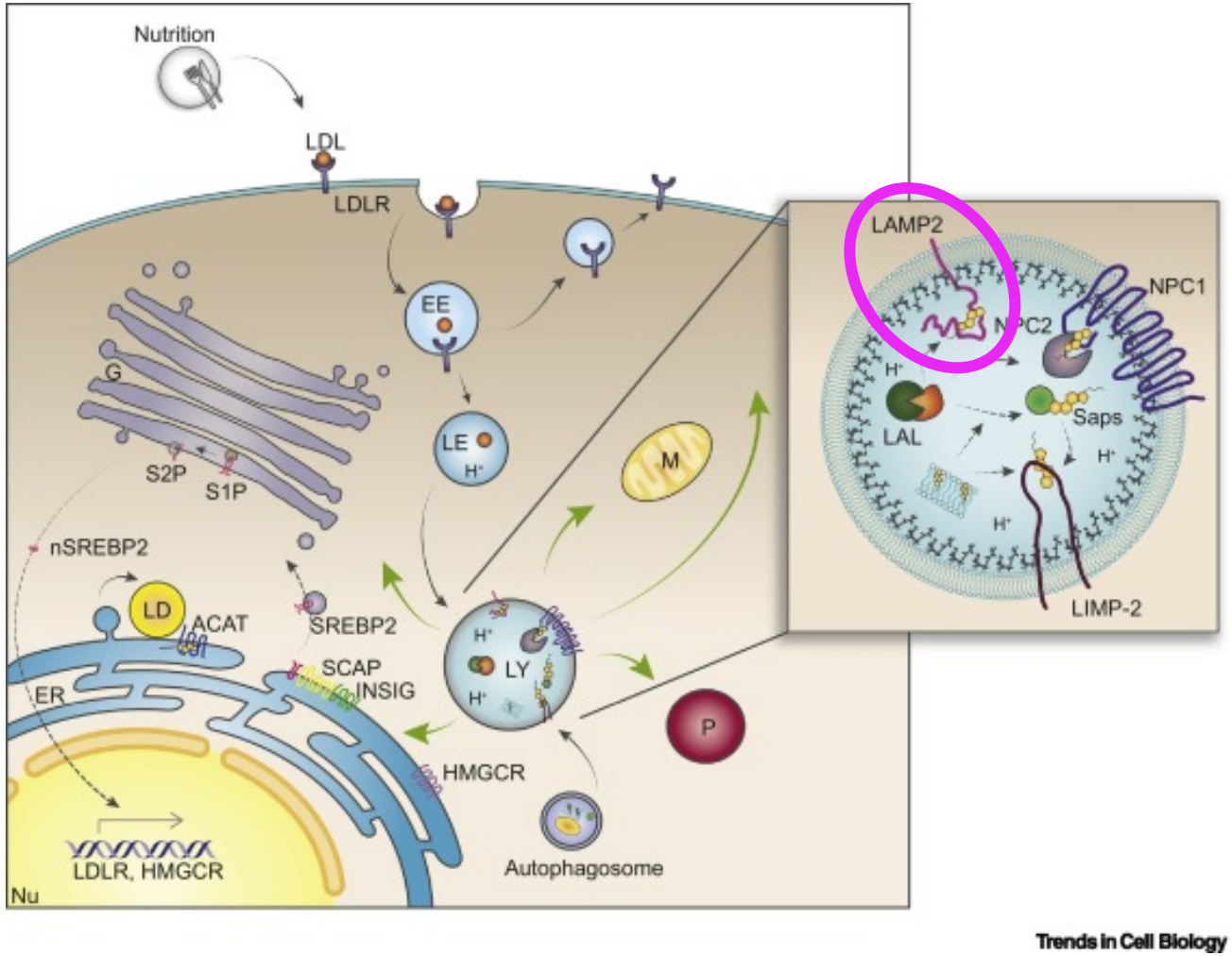
Volpatti, 2019, Disease Models & Mechanisms



Meng, 2020, Trends in Cell Biology

Identity of cellular compartments:

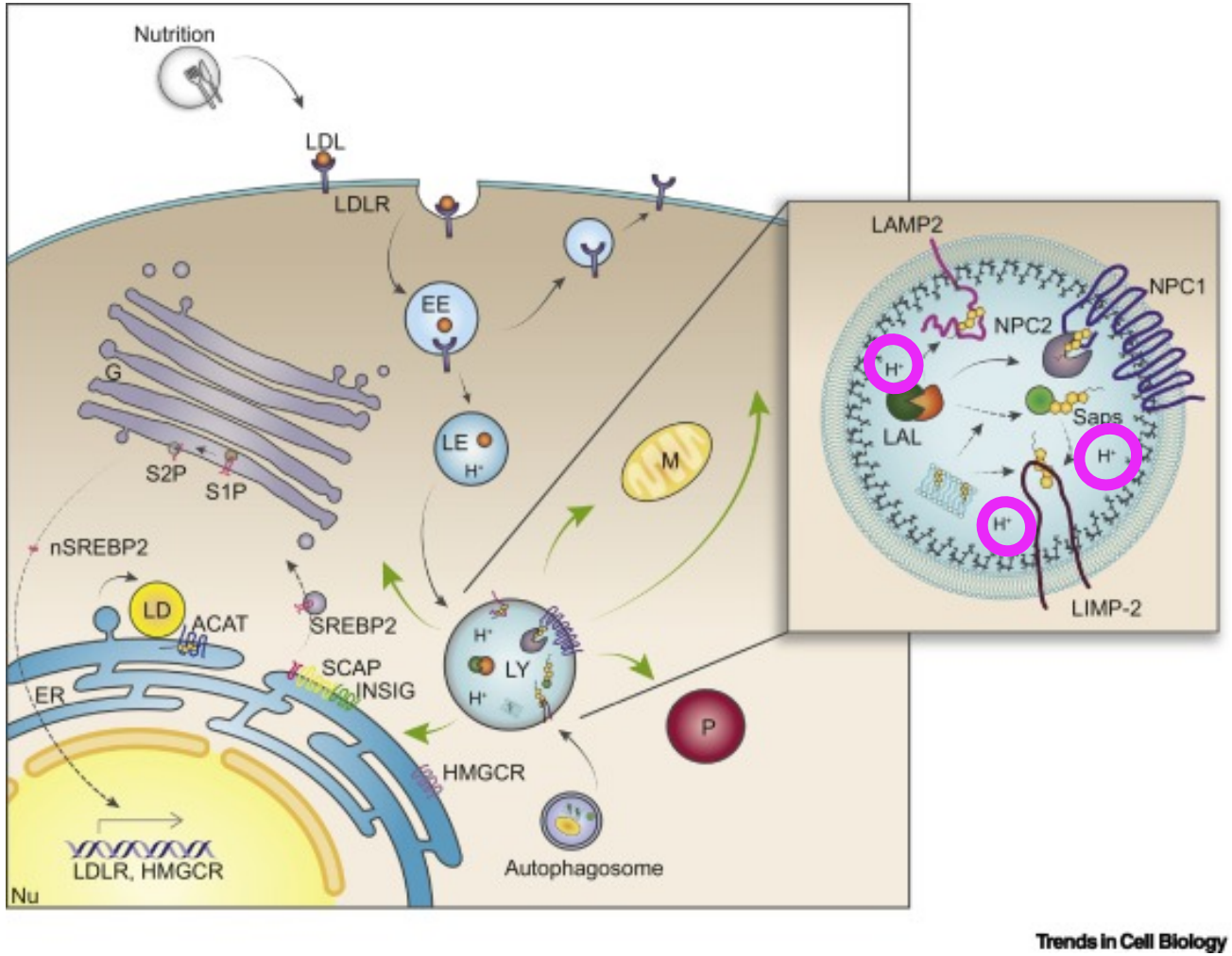
- Lipid composition
- Membrane proteins,
  e.g. LAMP1 & 2 in lysosomes



Meng, 2020, Trends in Cell Biology

Identity of cellular compartments:

- Lipid composition
- Membrane proteins
- Physicochemical properties, e.g. membrane potential, pH



Meng, 2020, Trends in Cell Biology

GDCh Communications Angewandte International Edition Chemie

Photoactivatable Molecules

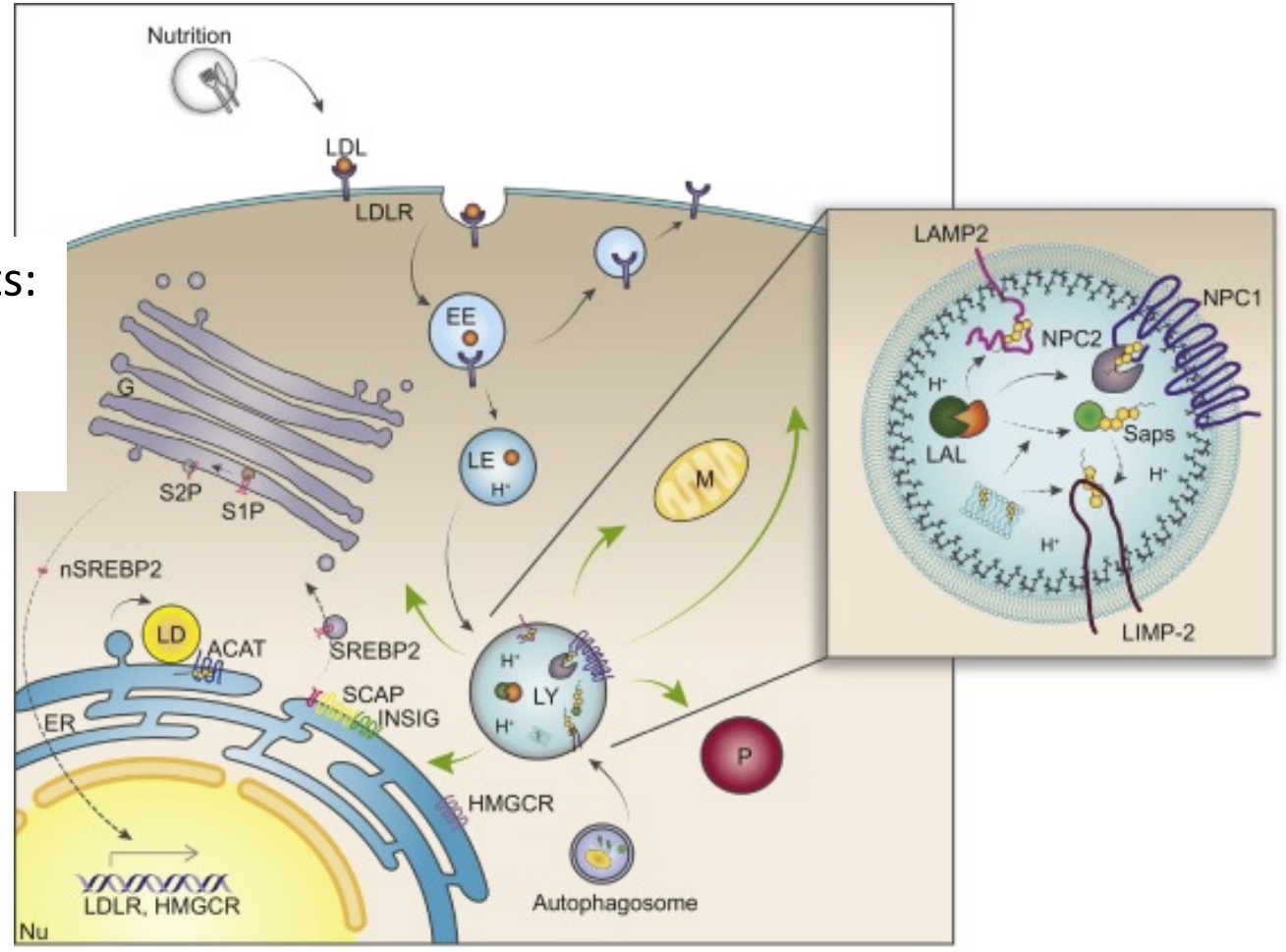
International Edition: DOI: 10.1002/anie.201807497
German Edition: DOI: 10.1002/ange.201807497

A Click Cage: Organelle-Specific Uncaging of Lipid Messengers

*Nicolai Wagner, Milena Stephan, Doris Höglinger, and André Nadler**

Specific manipulation of cellular compartments:

- Lipid composition
- Membrane proteins
- Physicochemical properties



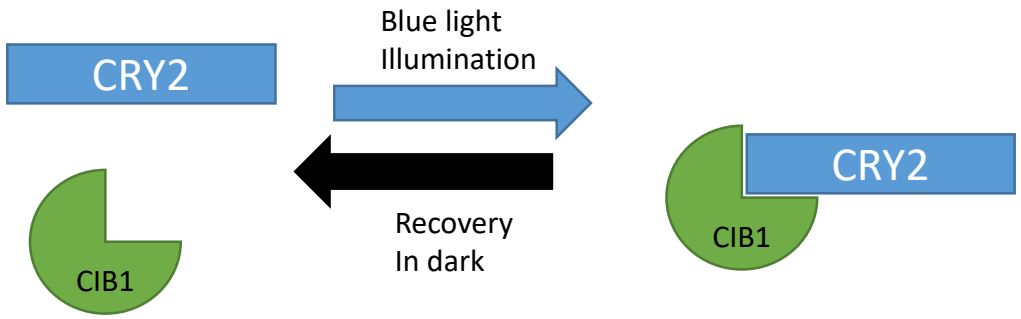
nature chemical biology ARTICLE
PUBLISHED ONLINE: 11 APRIL 2016 | DOI: 10.1038/NCHEMBIO.2064

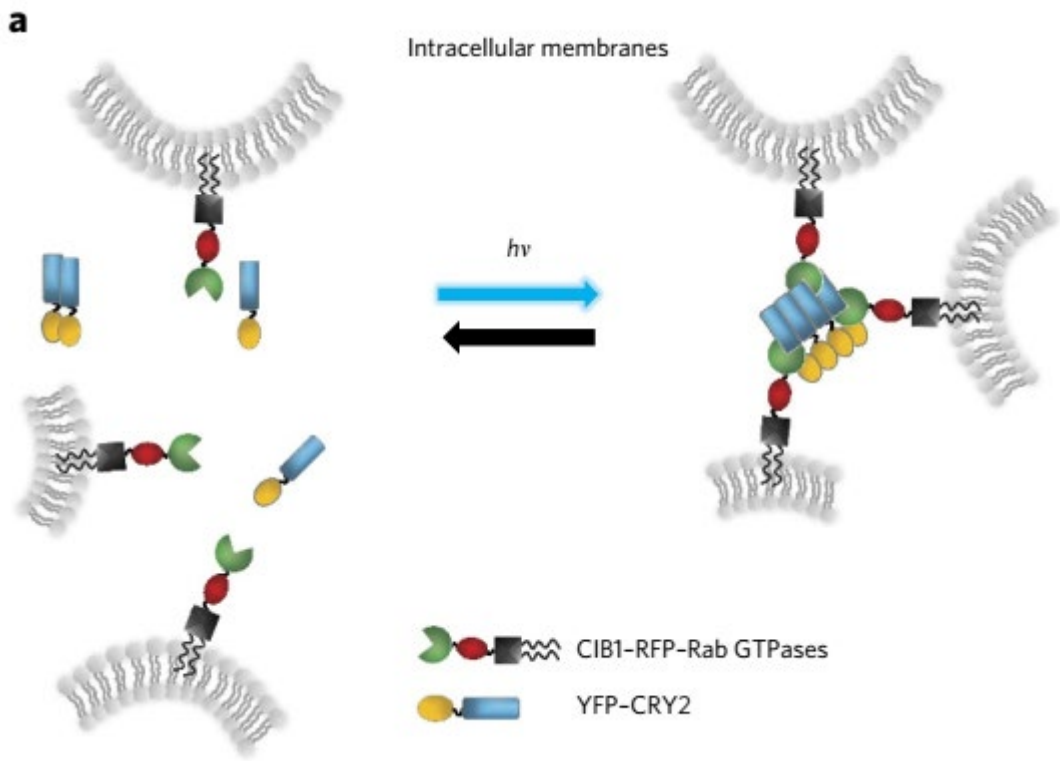
Optogenetic oligomerization of Rab GTPases regulates intracellular membrane trafficking

Mai Khanh Nguyen[1], Cha Yeon Kim[2], Jin Man Kim[3], Byung Ouk Park[4], Sangkyu Lee[4], Hyerim Park[1] & Won Do Heo[1,4,5]*

Light-activated reversible inhibition by assembled trap of intracellular membranes (IM-LARIAT)

CRY2 and CIB1 bind upon blue light illumination





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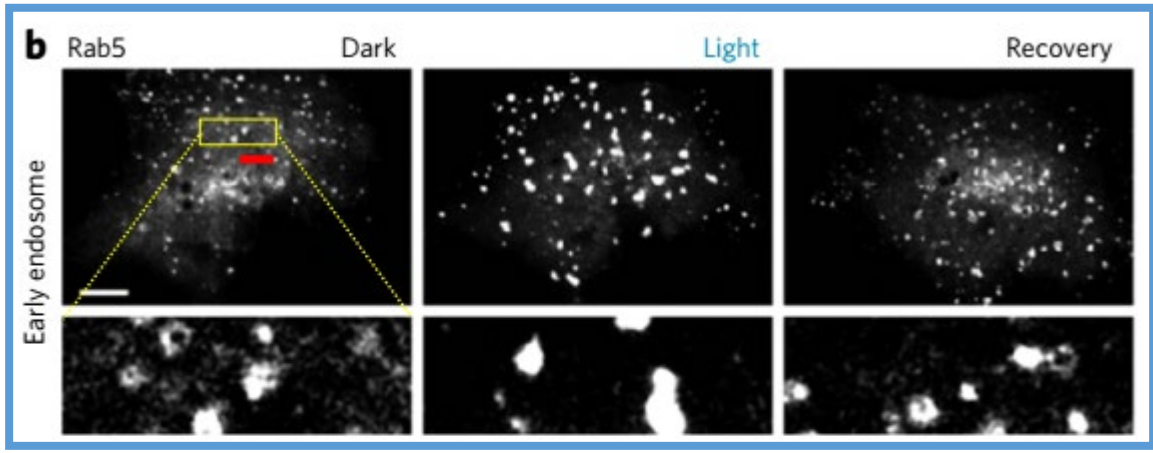
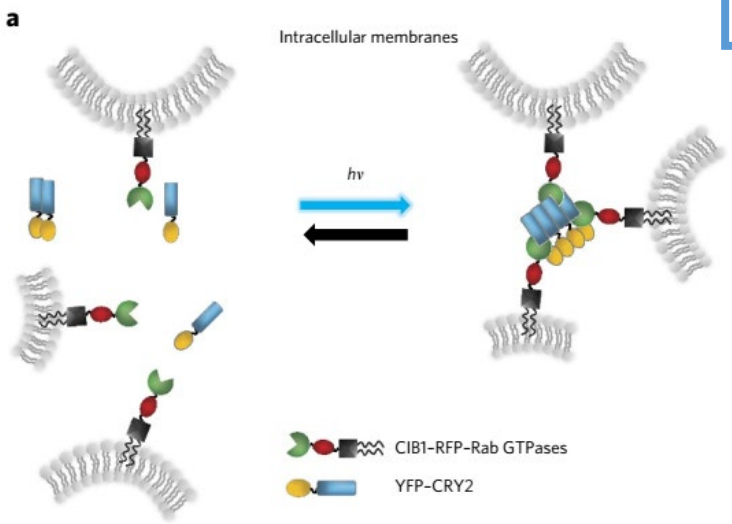
Coupling GTPases to CIB1

Illumination = sequestration of membranes
& aggregation of vesicles





Coupling GTPases to CIB1

Illumination = sequestration of membranes
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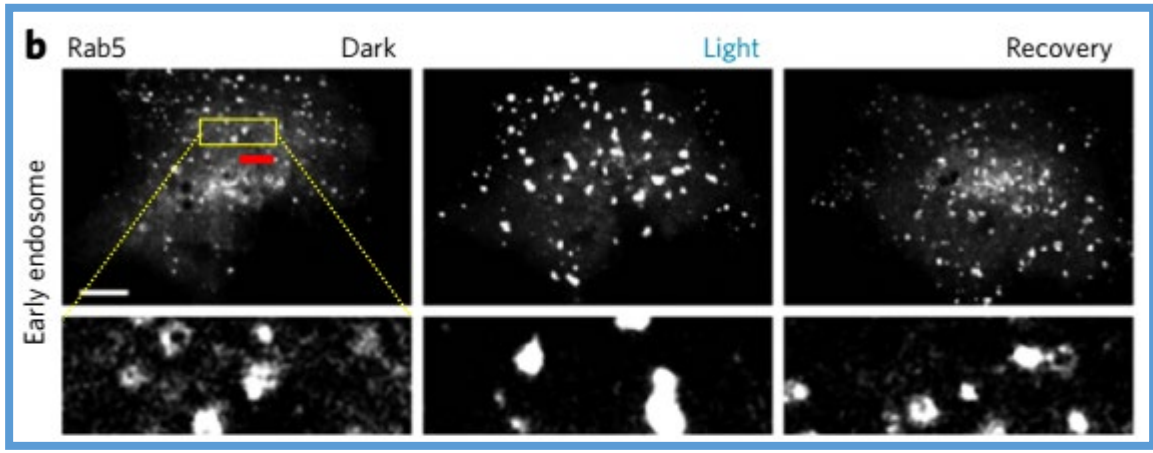
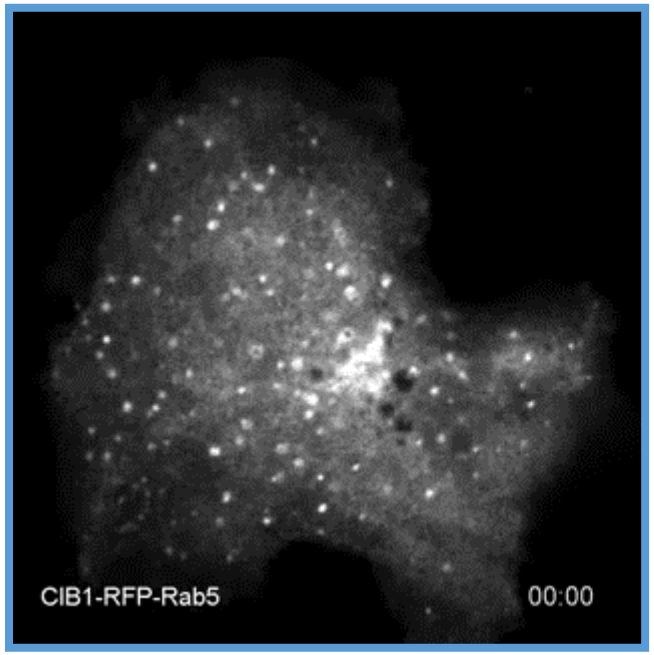
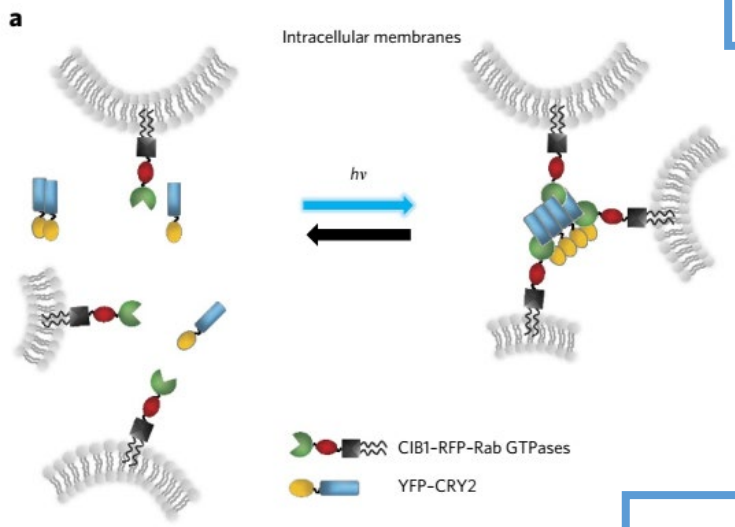
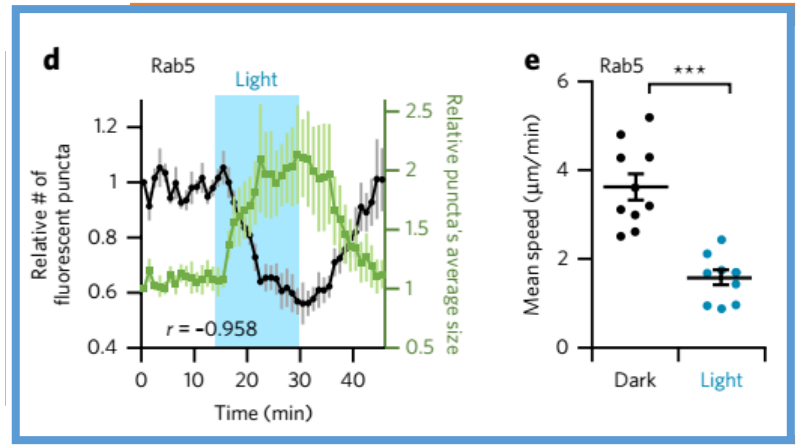
- Reduced movement (1.55 um/min vs. 3.66 um/min)
- Increase in size (2.1x)
- Decrease in number (43%)









Coupling GTPases to CIB1

Illumination = sequestration of membranes
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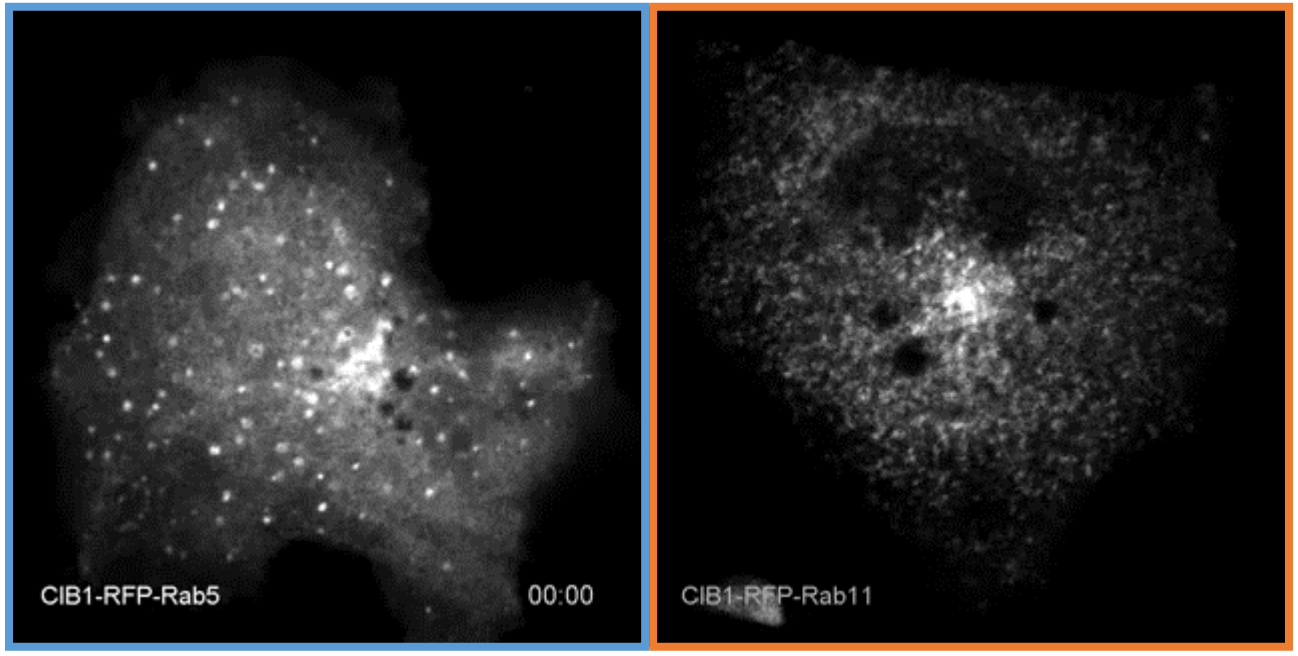
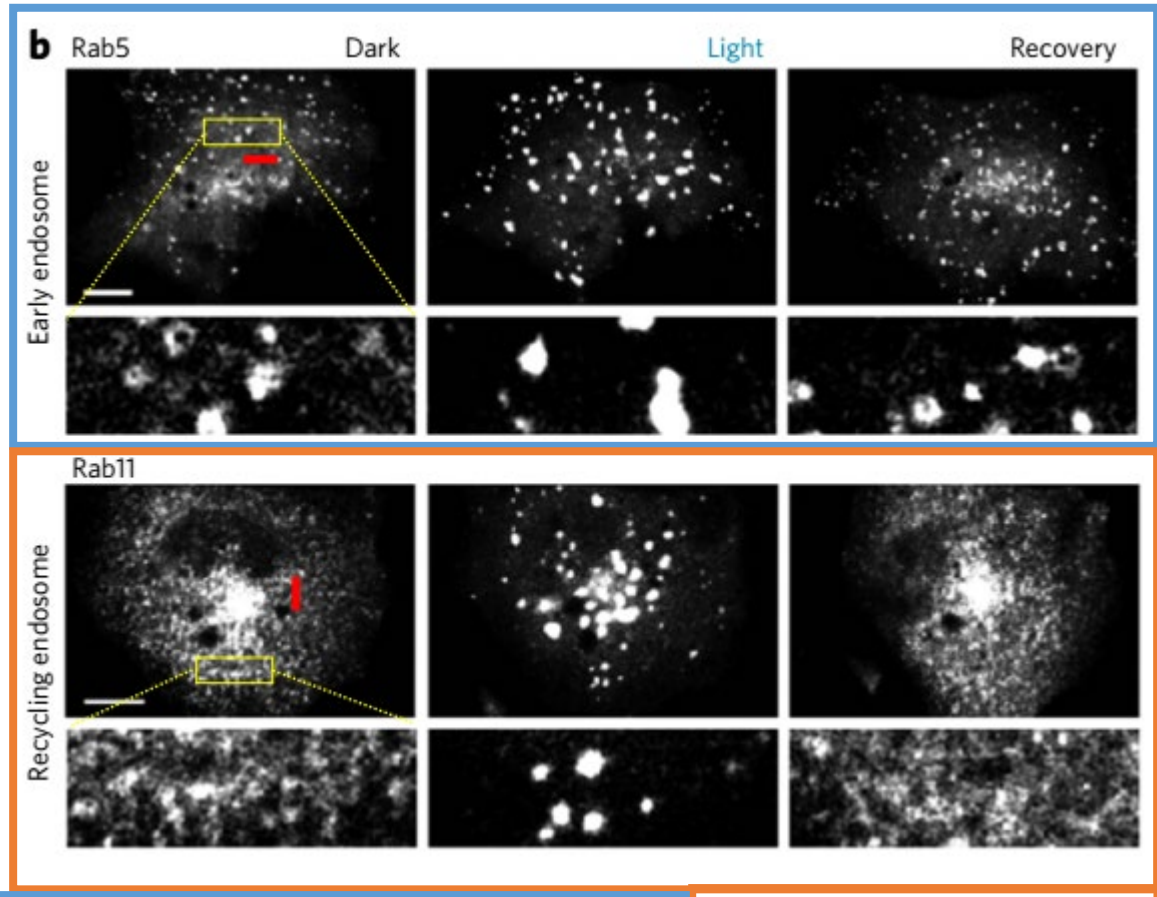
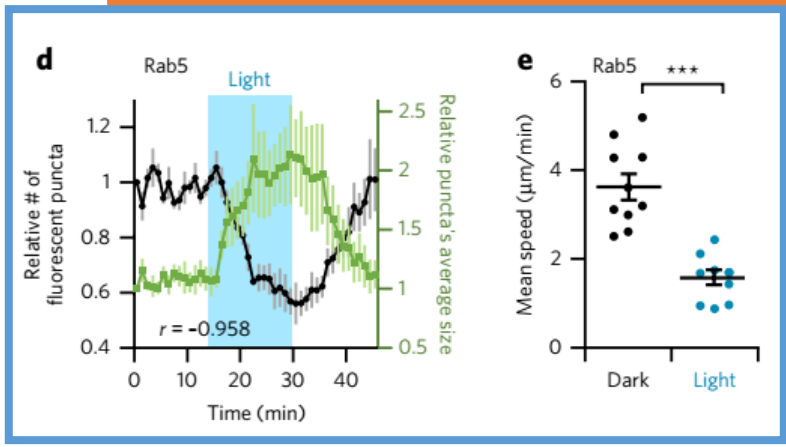
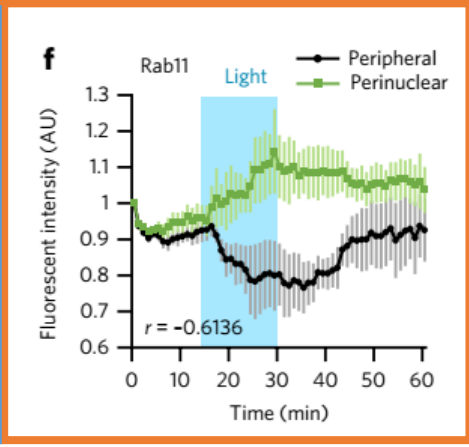
- Reduced movement (1.55 um/min vs. 3.66 um/min)
- Increase in size (2.1x)
- Decrease in number (43%)
- Withdrawl from periphery → perinuclear



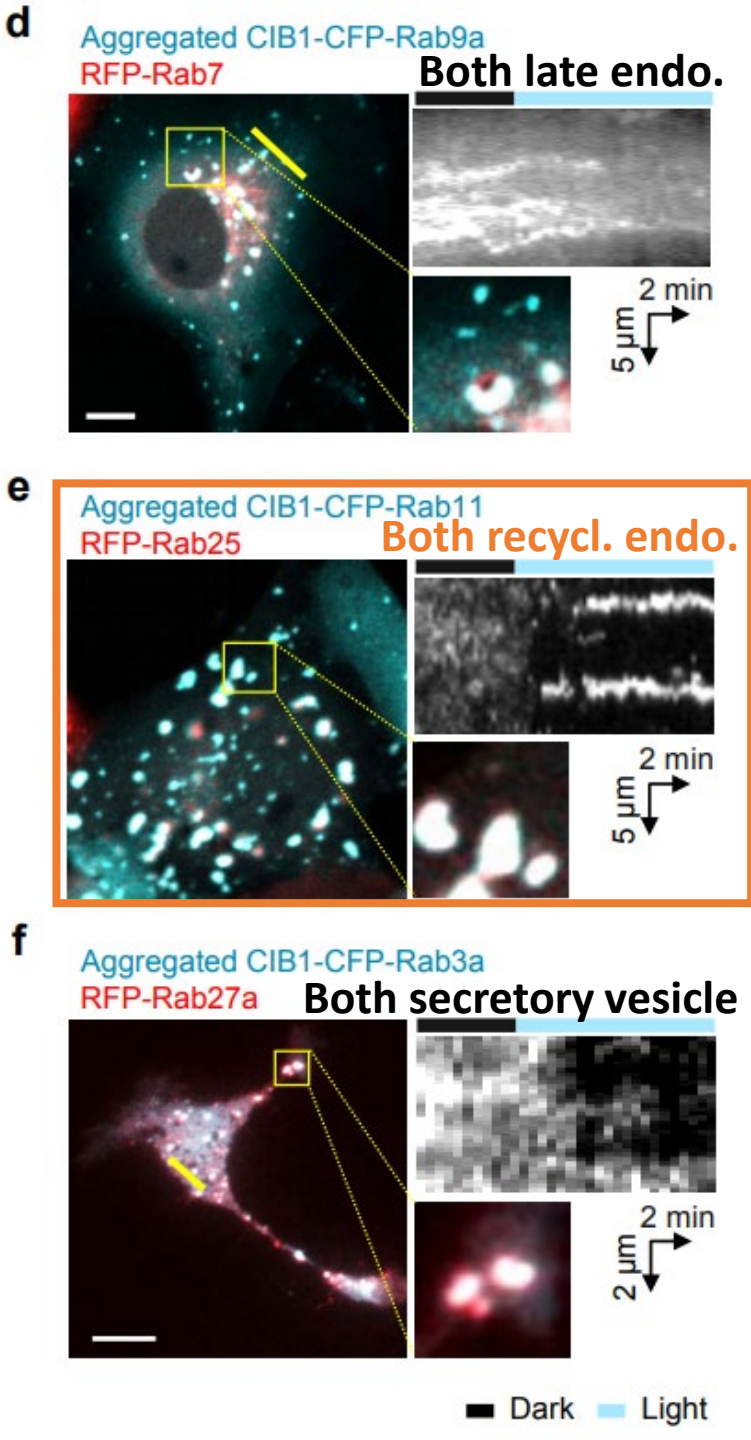






Specificity

- Rab 5: early endosomes
- **Rab11 & 25: recycling endosomes**
- **Rab7 & 9: late endosomal compartment**
- **Rab3a & 27a: secretory vesicles**
- Rab2a: ER-to-Golgi vesicles
- Rab6a: Golgi-to-PM vesicles



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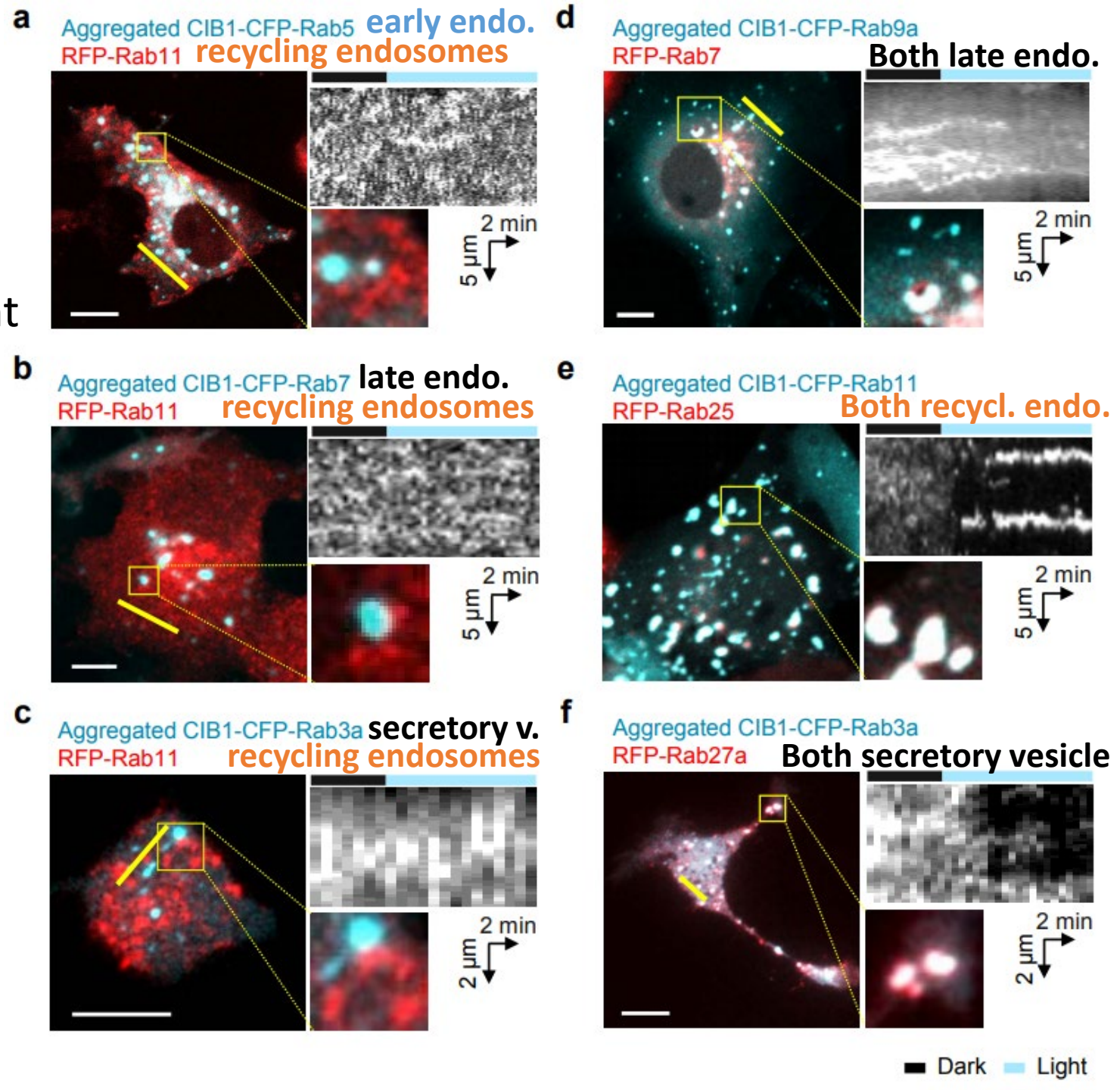


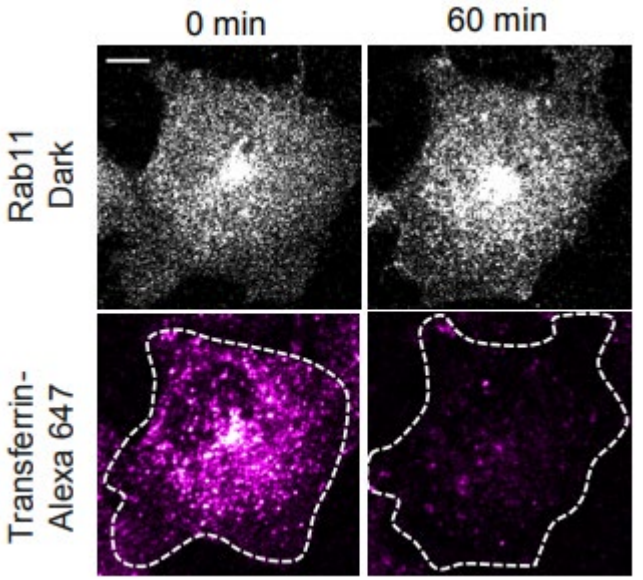
Optogenetic control of functional trafficking processes

→Demonstration of functional alteration upon rab-sequestration

Example1:

Transferrin recycling
=classical function of Rab11

Control: Transferrin recycling in COS-7 cells overexpressing CIB1–Rab11 (dark, inactive)



Rab7 = late endo
Rab11 = recycling endo

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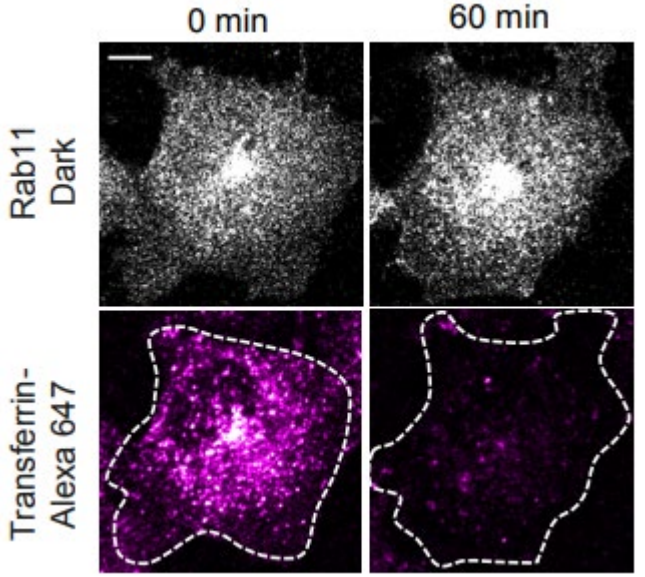
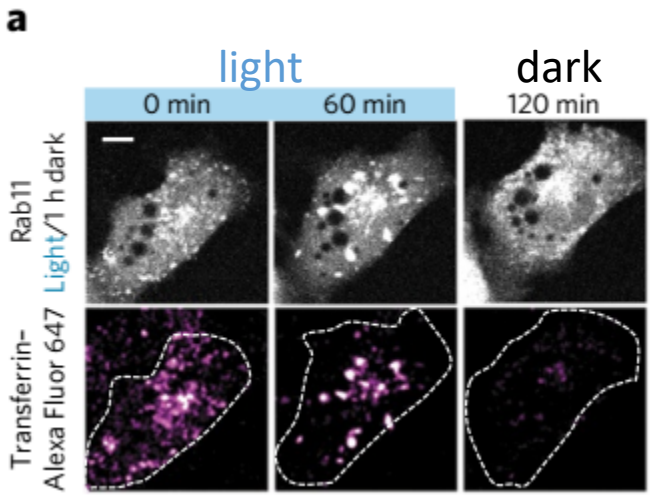
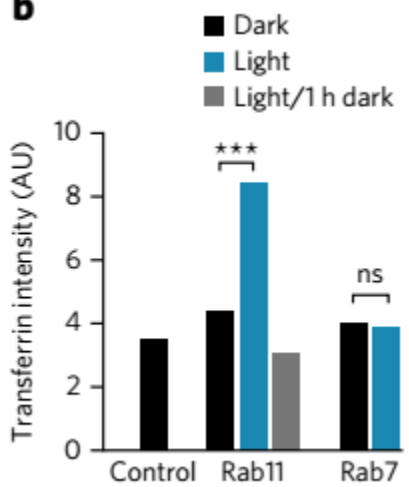




Live cell imaging



Fixed at end-point

Rab7 = late endo
Rab11 = recycling endo

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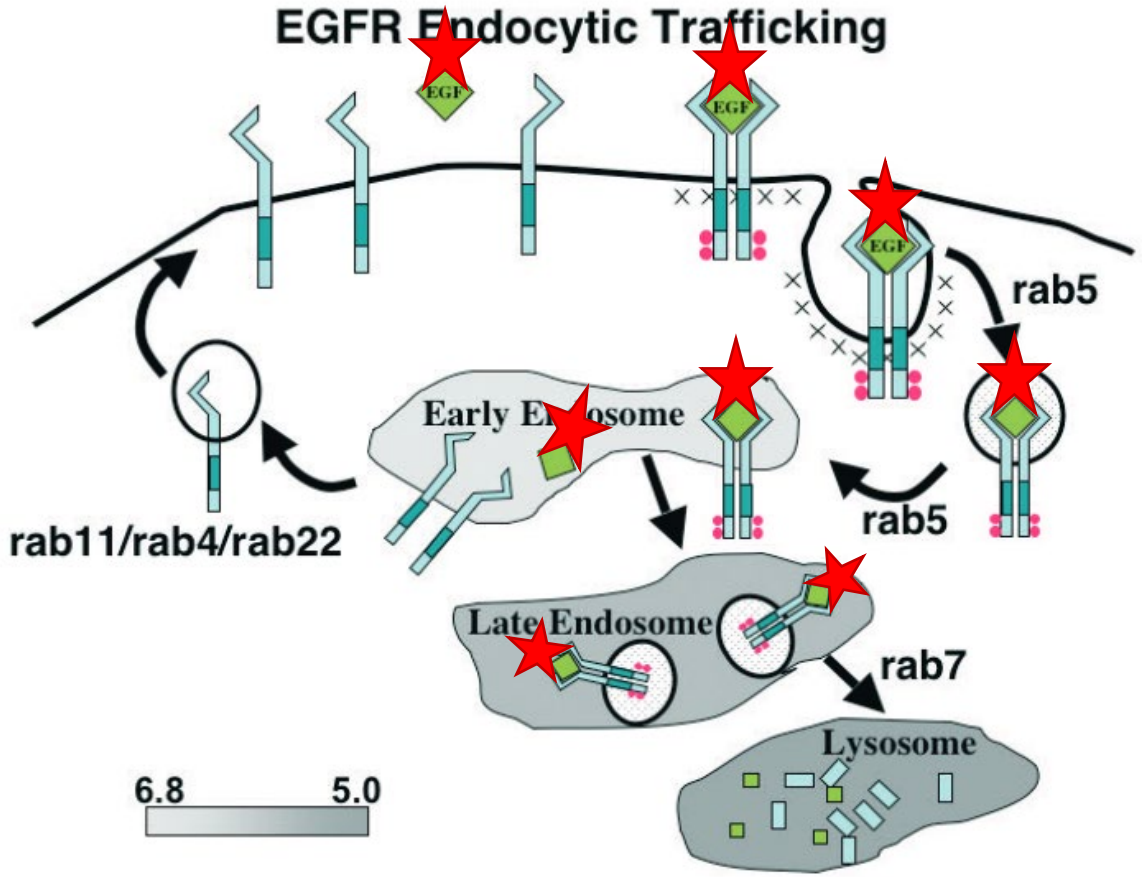
Example2:

Activation of cell surface receptors,
e.g. EGFR endocytosis involving Rab5

And Rab7-mediated degradation



Ceresa, 2006, Histol Histopathol 21: 987-993

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Optogenetic control of functional trafficking processes

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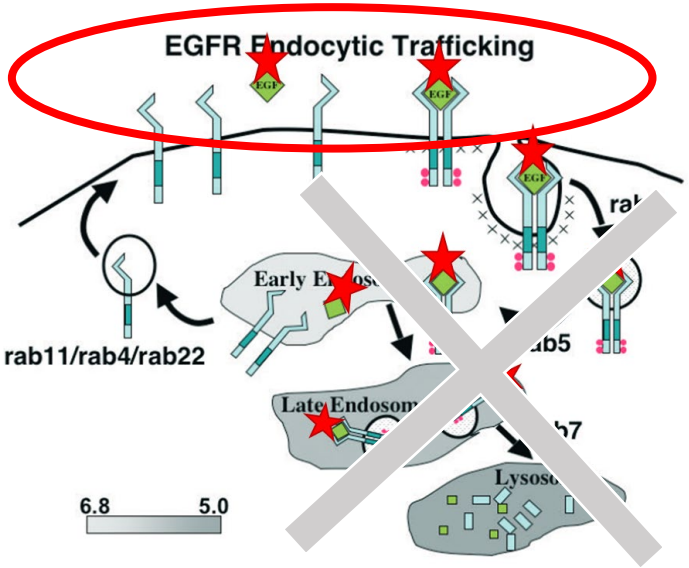

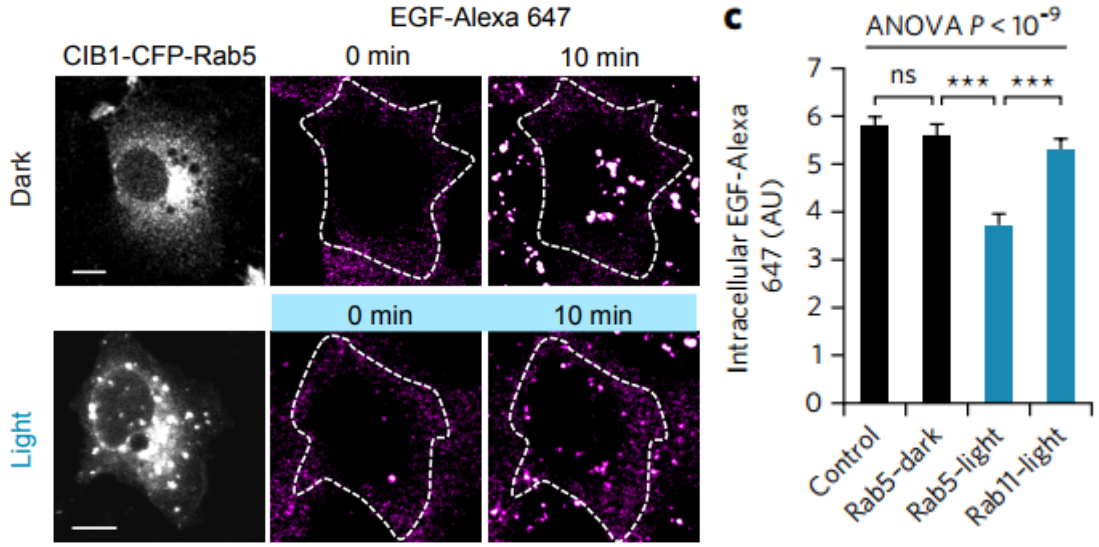

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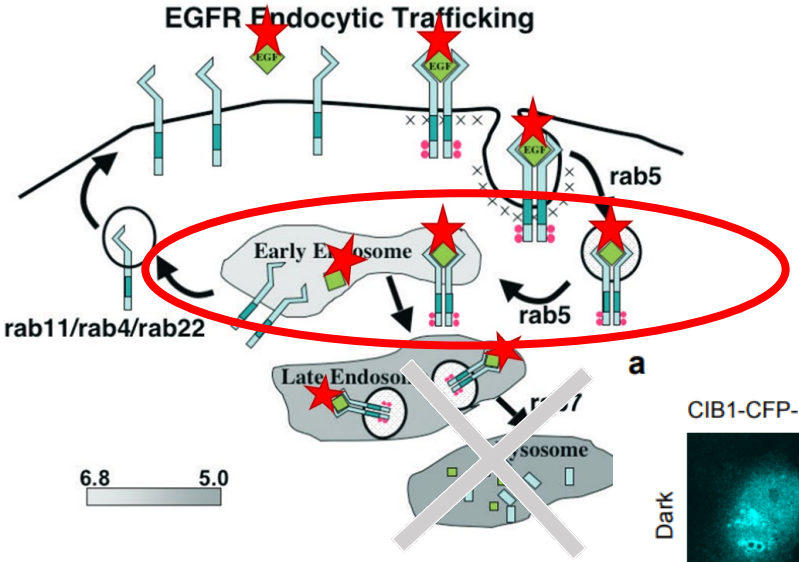

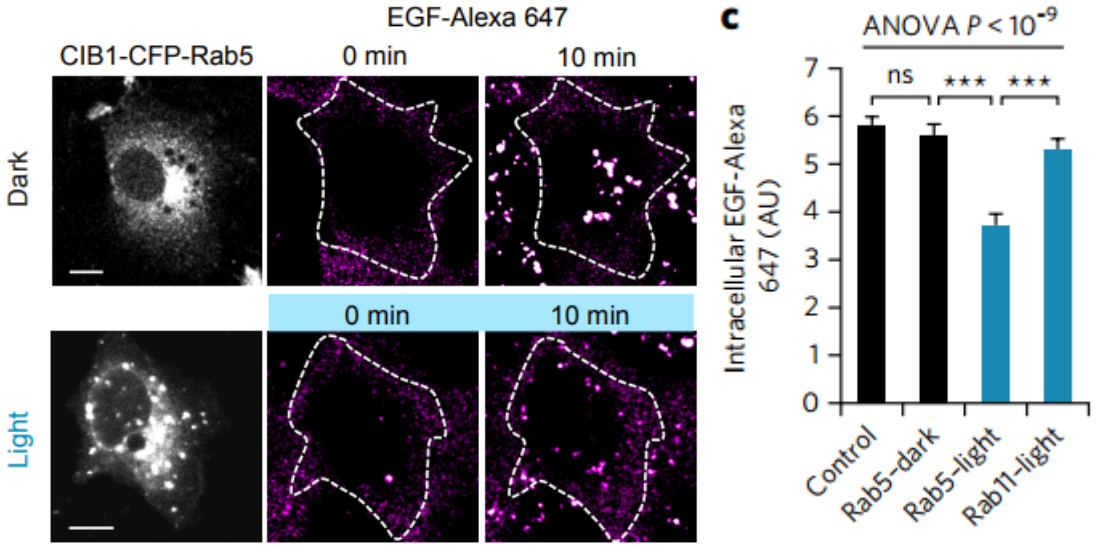

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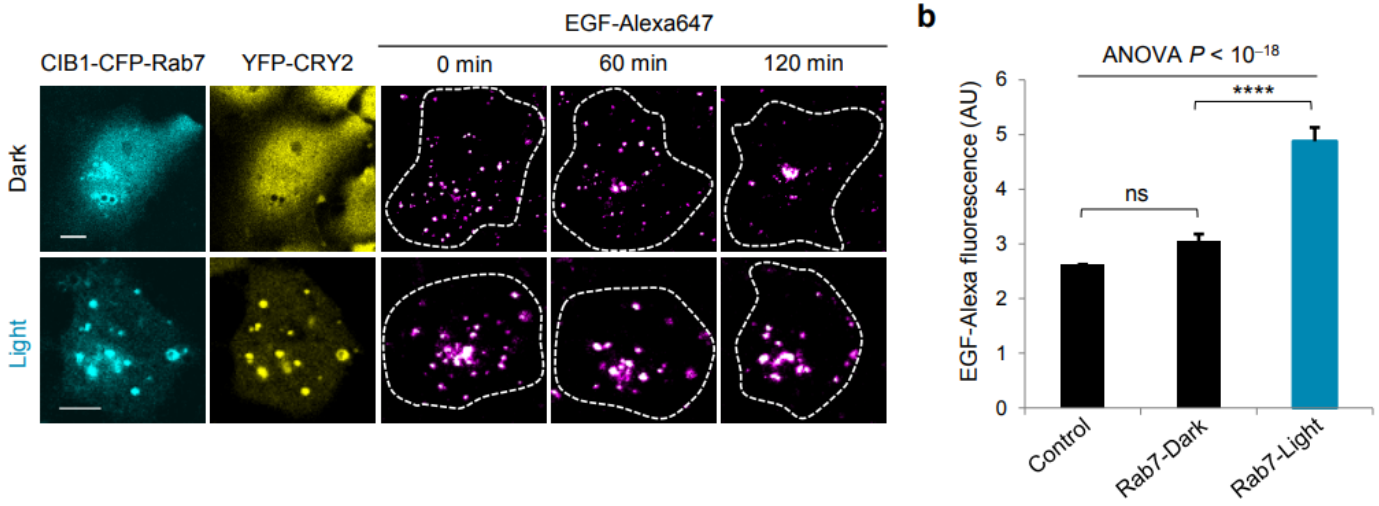
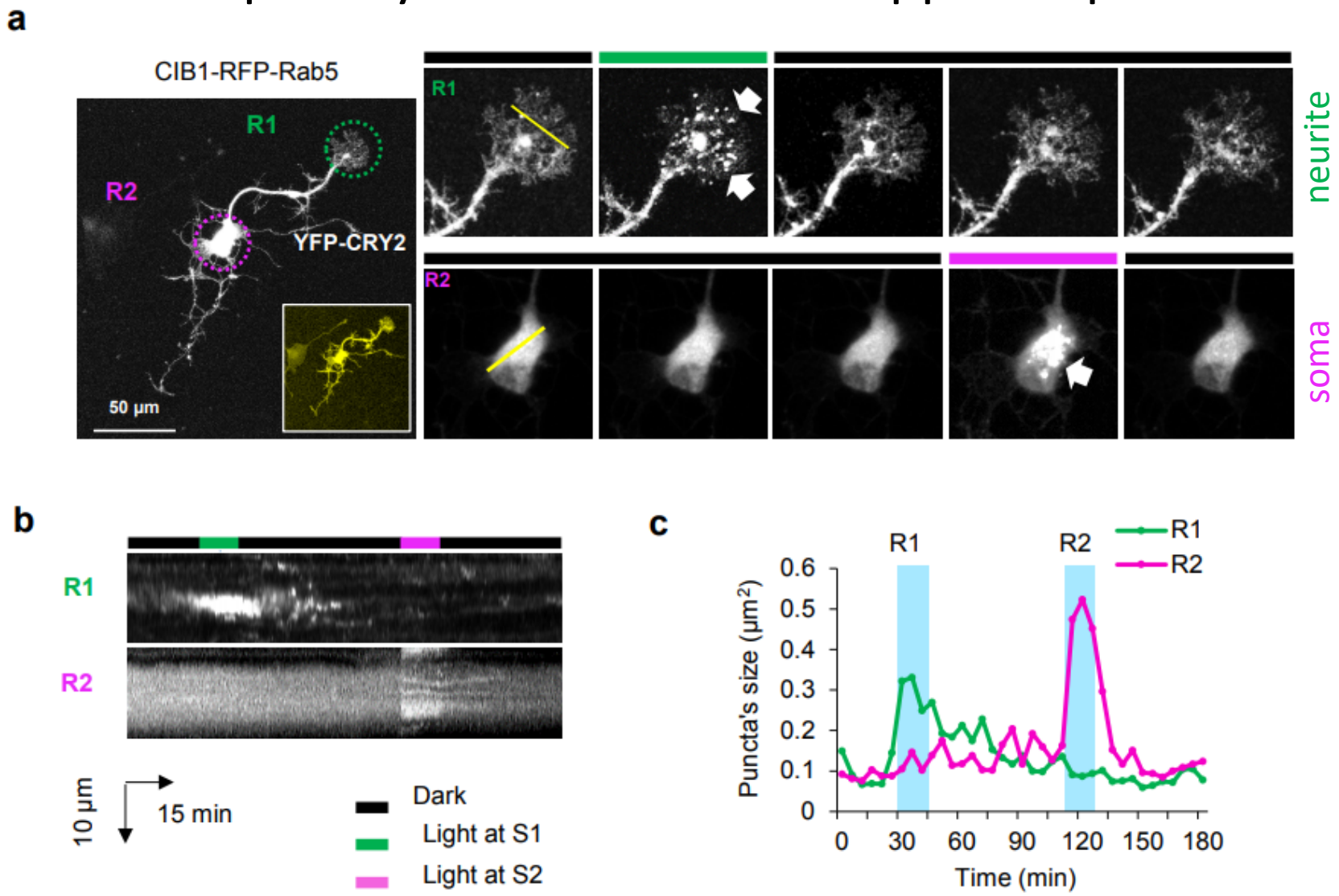


Rab5 = early endo
Rab7 = late endo
Rab11 = recycling endo

Spatial control of membrane trafficking pathways

→Demonstration of spatially defined effect in hippocampal neurons



Function of the GTPases in growth cones (GC) of neurons

→Existing literature suggests that:

- →**Rab5** functions in the recycling pathway, which is necessary for the elongation of neurites
- →**Rab11** is implicated in regulating the trafficking of integrin to adhesive points in the GCs, which is also necessary for neurite growth

→Does local aggregation of Rab5- or Rab11- targeted endosomes in the **soma or GCs** of young neurons (3-6 days *in vitro*) trigger a different neurite outgrowth pattern?

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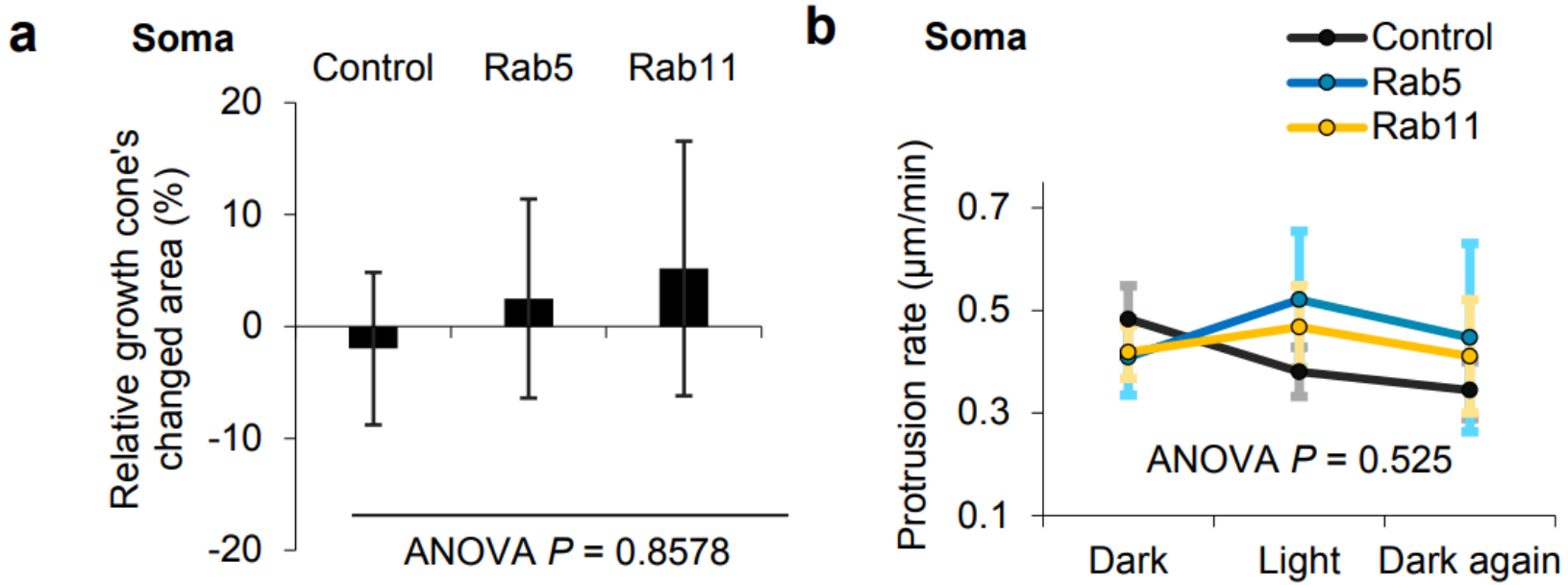

Rab5- or Rab11-targeted aggregation in the soma of hippocampal neurons does not interfere with instant protrusion and growth.

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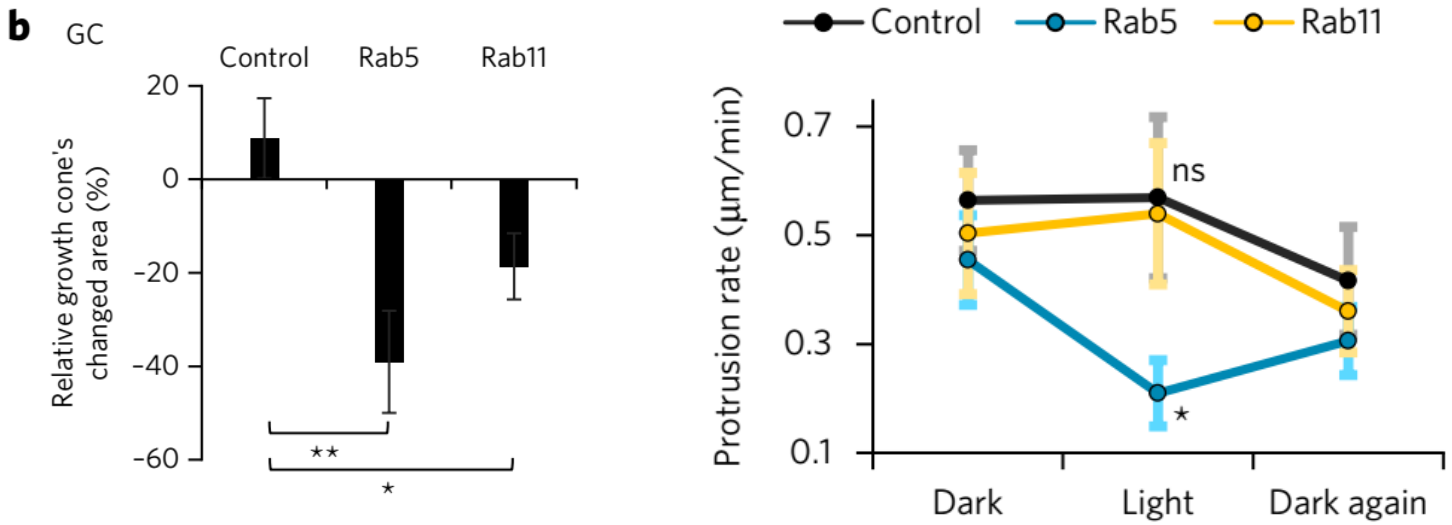
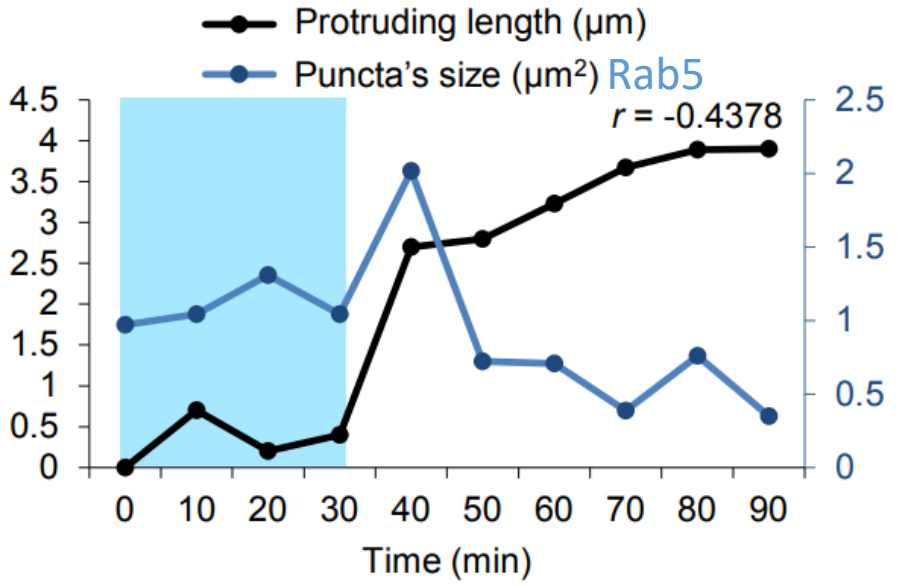
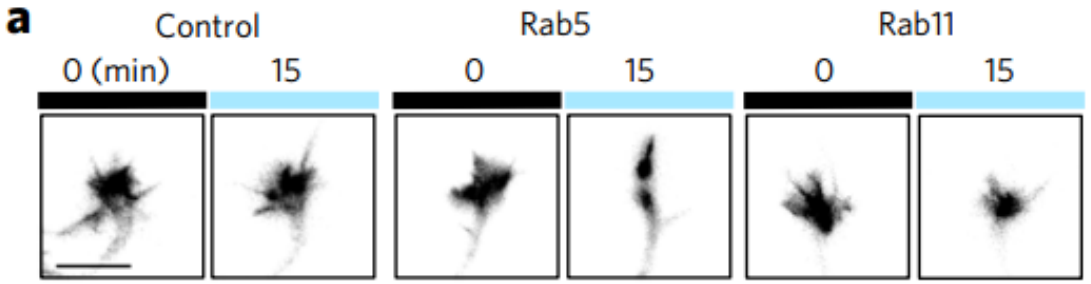
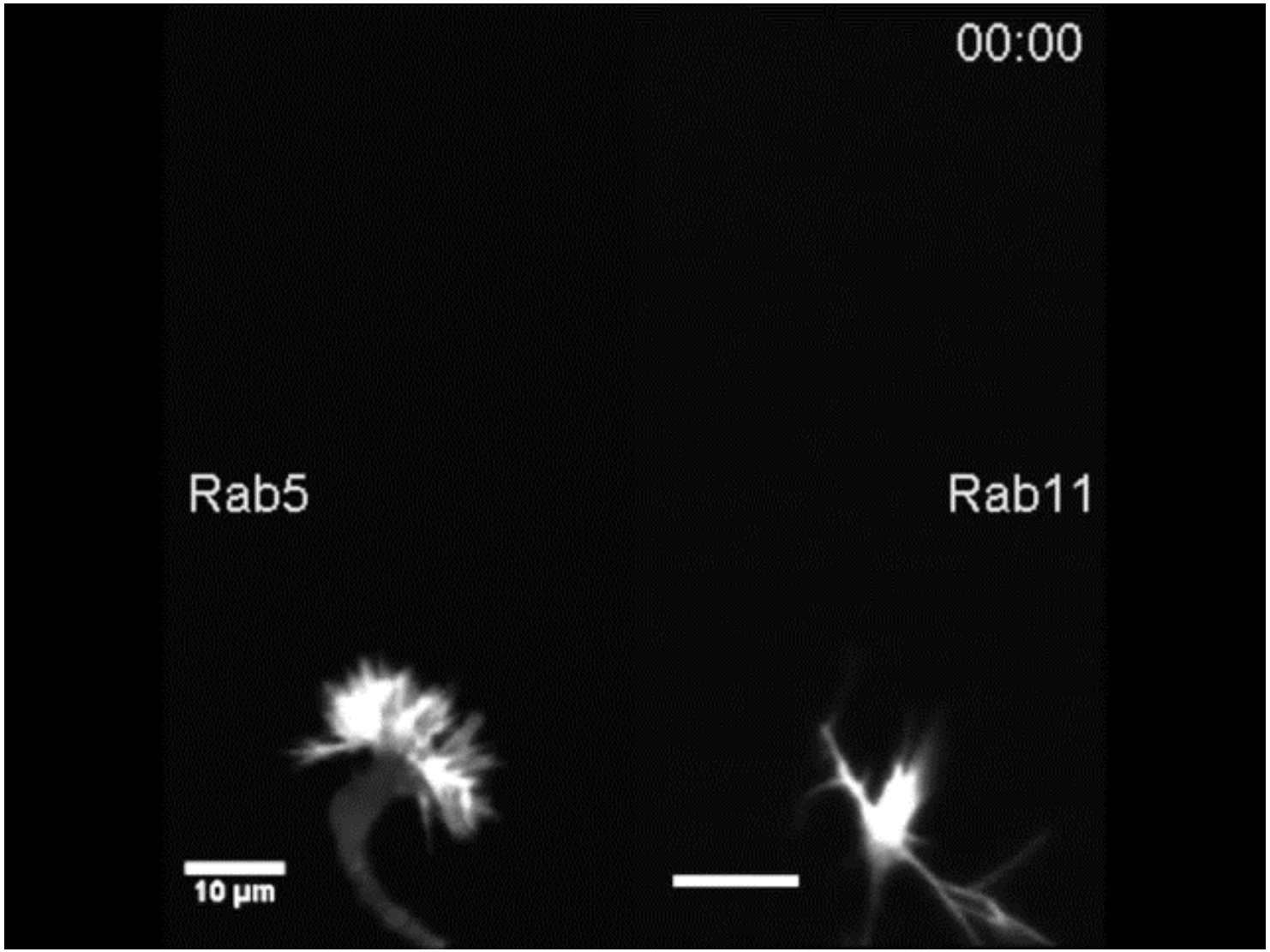






→ Rab5-compartments may affect the immediate growth rate through the rapid production of membranes
→ Rab11-targeted compartments may affect the stabilization of GCs and support dendritic growth over the long term



Summary
Light-activated reversible inhibition by assembled trap of intracellular membranes (IM-LARIAT)

- Sequestration of compartments
  – «Knock Down» / «Inhibition» of a whole cellular compartment
- Specific for distinct vesicular compartements of the cell
- Light-activated
  - Temporal control (light vs dark)
  - Reversible
  - Spatial control (e.g. soma vs. neurite)

GDCh Communications Angewandte International Edition Chemie

Photoactivatable Molecules

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A Click Cage: Organelle-Specific Uncaging of Lipid Messengers

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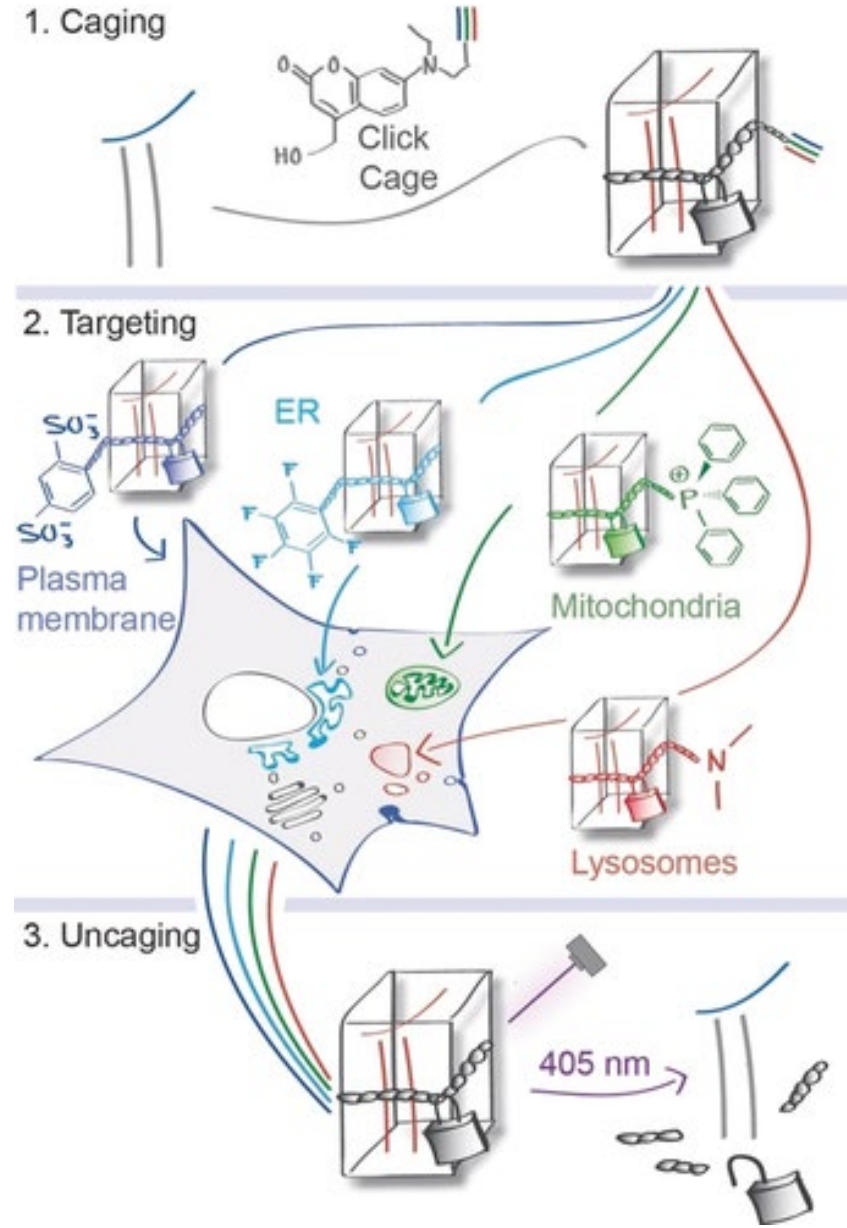
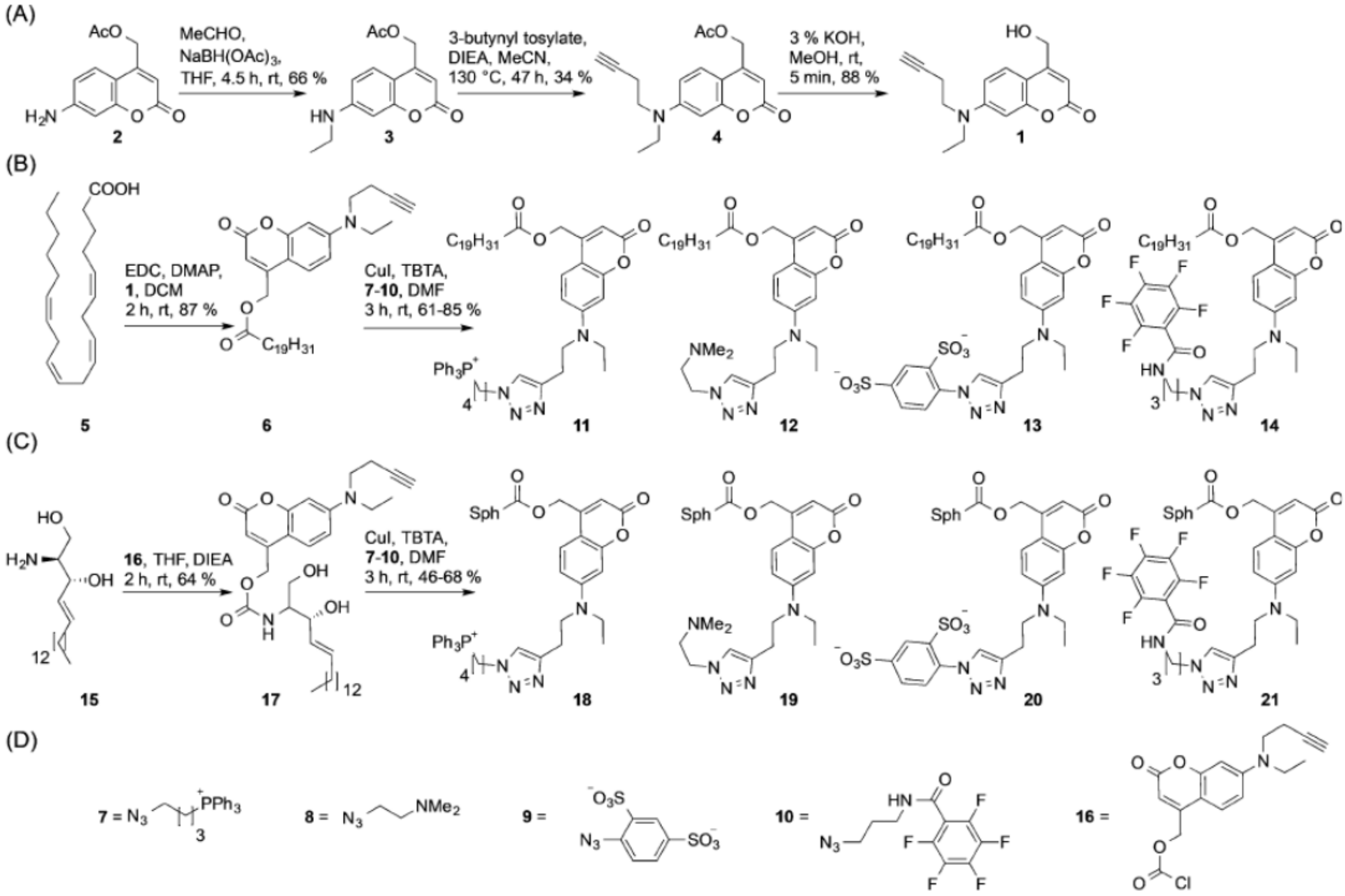


Background / General Idea

- Most available lipid messengers are not organelle specific
  - Endogenous messengers for cellular processes → manipulation
  - Fluorescence-tag for identification → visualization

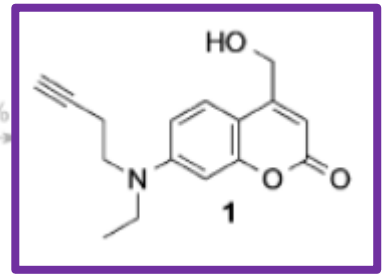
- For organelle specificity, many probes with different properties need to be generated

→ «Toolbox» using click-chemistry can provide this easier


(A)
MeCHO, NaBH(OAc)3, THF, 4.5 h, rt, 66 %
3-butynyl tosylate, DIEA, MeCN, 130 °C, 47 h, 34 %
3 % KOH, MeOH, rt, 5 min, 88 %
2
3
4
1
(B)
EDC, DMAP, 1, DCM 2 h, rt, 87 %
CuI, TBTA, 7-10, DMF 3 h, rt, 61-85 %
5
6
11
12
13
14
(C)
16, THF, DIEA 2 h, rt, 64 %
CuI, TBTA, 7-10, DMF 3 h, rt, 46-68 %
15
17
18
19
20
21
(D)
7 =
8 =
9 =
10 =
16 =

Principle

«Click Cage Cumarin» was chemically modified for the respective target organelle



- Click Cage Cumarin was attached to
  - Arachidonic acid
  - Sphingosine

- Organelle specific modifications
  - cationic triphenylphosphonium azide for the mitochondrial probe
  - tertiary amino azide for the lysosomal probe
  - sulfonated azide for the plasma-membrane-specific probe
  - perfluorinated azide for the ER probe

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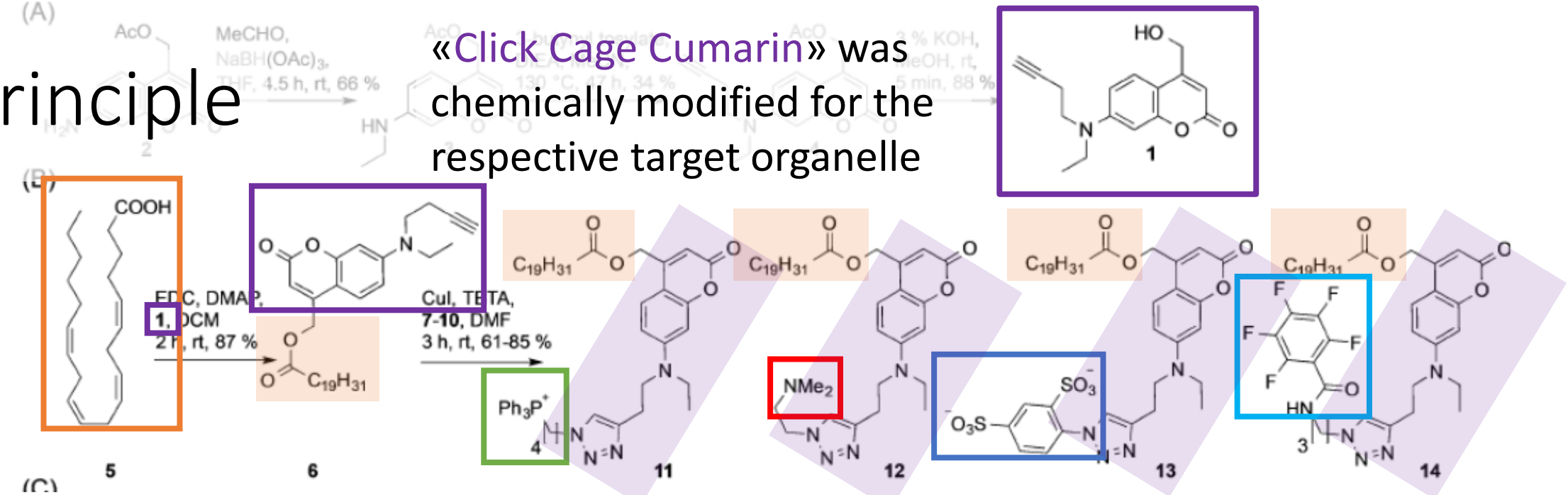

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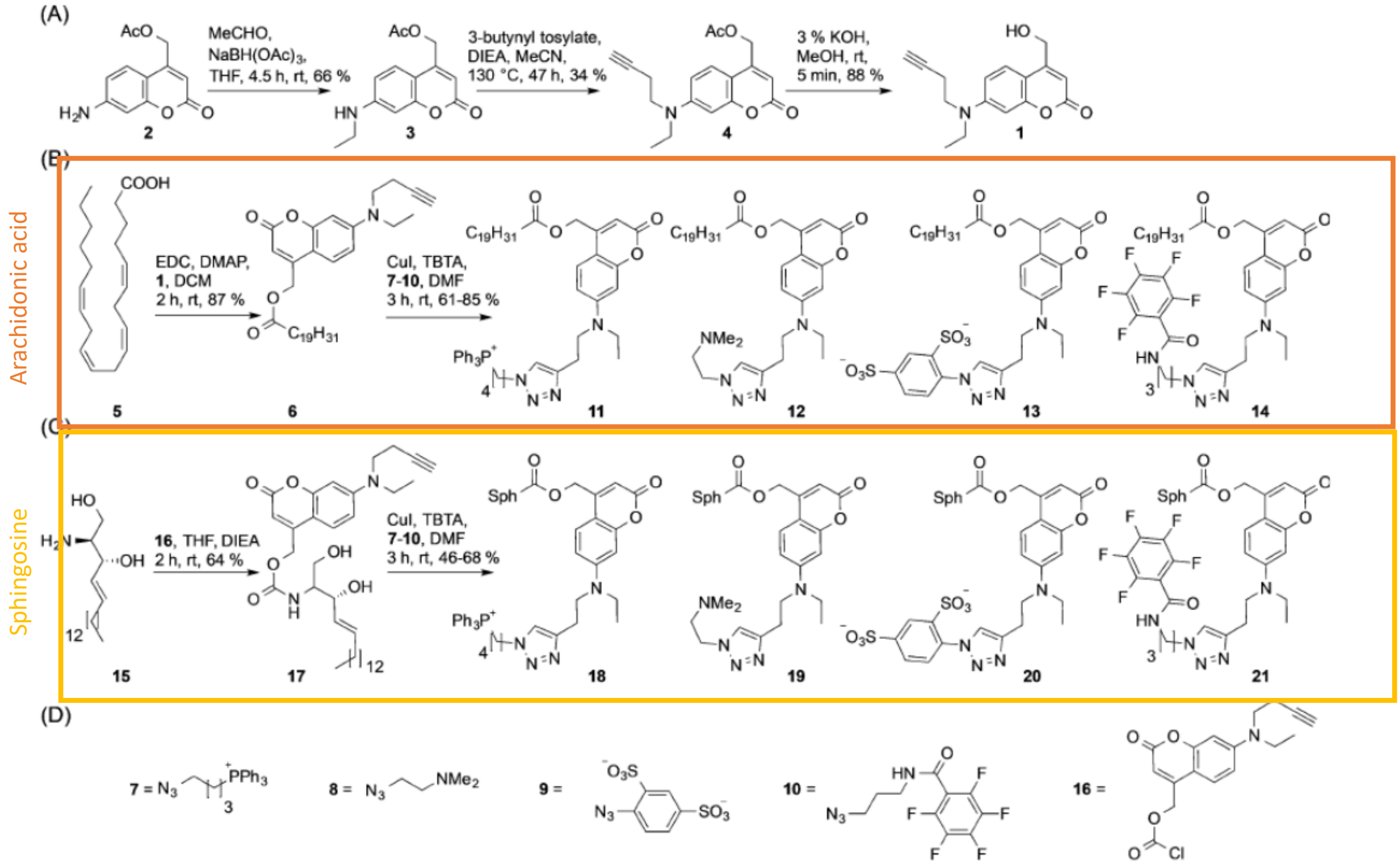
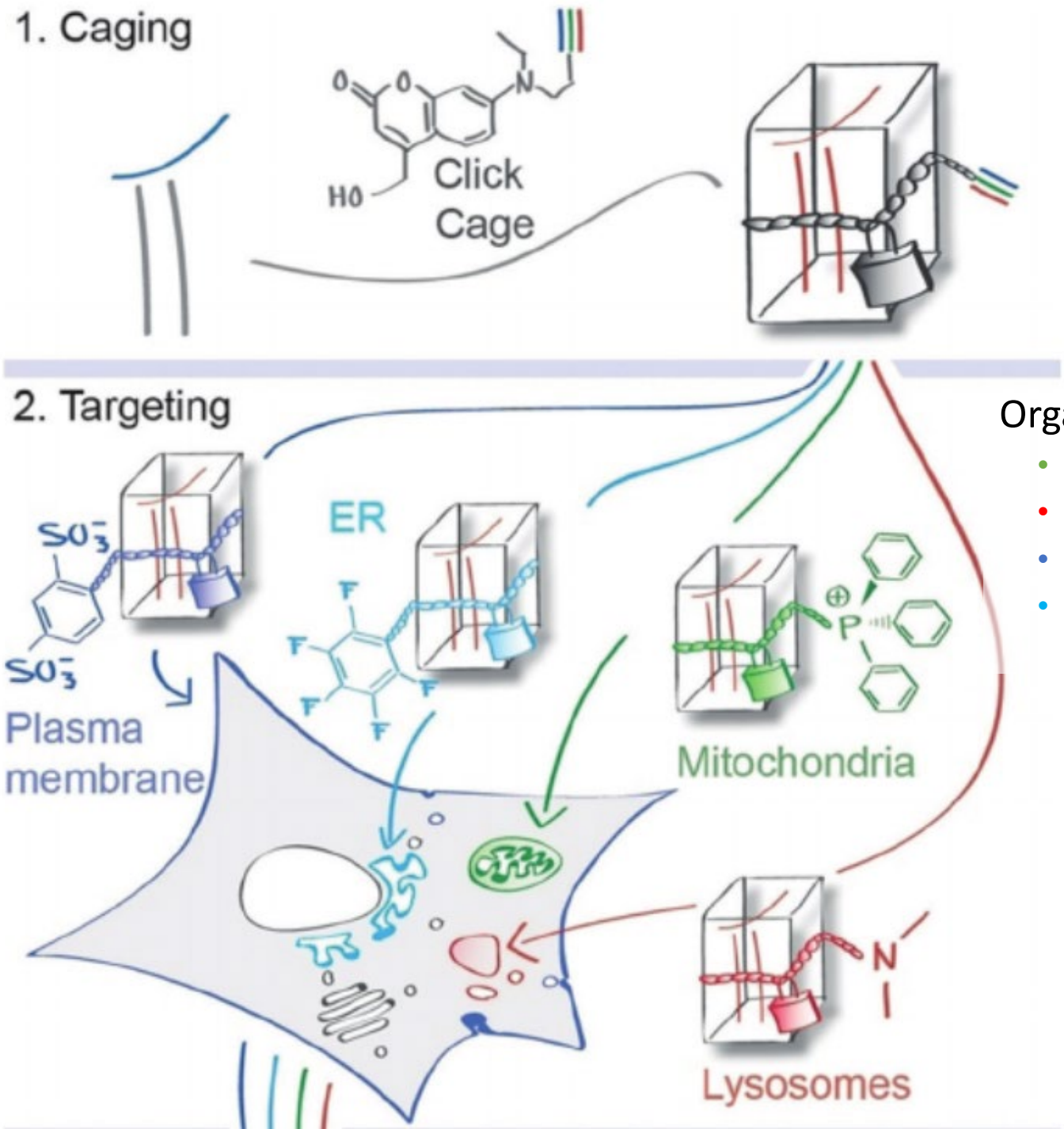
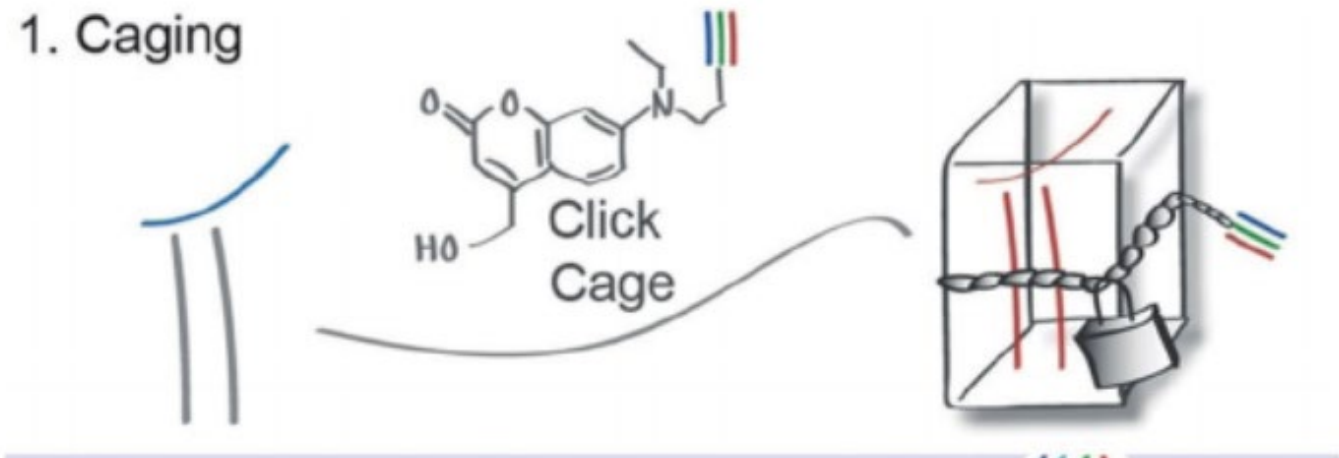
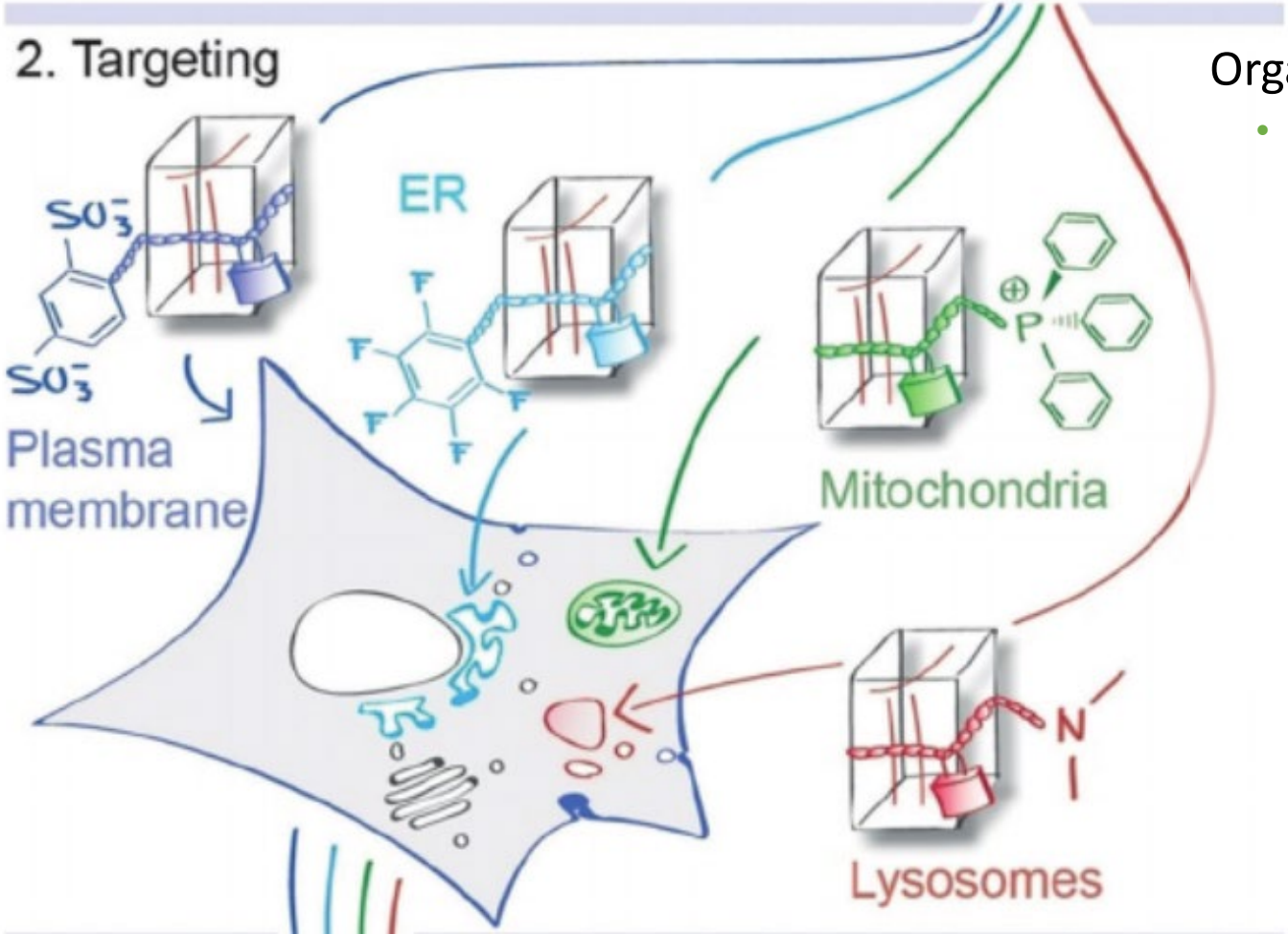

(A)
MeCHO, NaBH(OAc)3, THF, 4.5 h, rt, 66 %
3-butynyl tosylate, DIEA, MeCN, 130 °C, 47 h, 34 %
3 % KOH, MeOH, rt, 5 min, 88 %
2
3
4
1
(B)
Arachidonic acid
EDC, DMAP, 1, DCM 2 h, rt, 87 %
CuI, TBTA, 7-10, DMF 3 h, rt, 61-85 %
5
6
11
12
13
14
(C)
Sphingosine
16, THF, DIEA 2 h, rt, 64 %
CuI, TBTA, 7-10, DMF 3 h, rt, 46-68 %
15
17
18
19
20
21
(D)
7 =
8 =
9 =
10 =
16 =



Organelle specific modifications via click chemistry

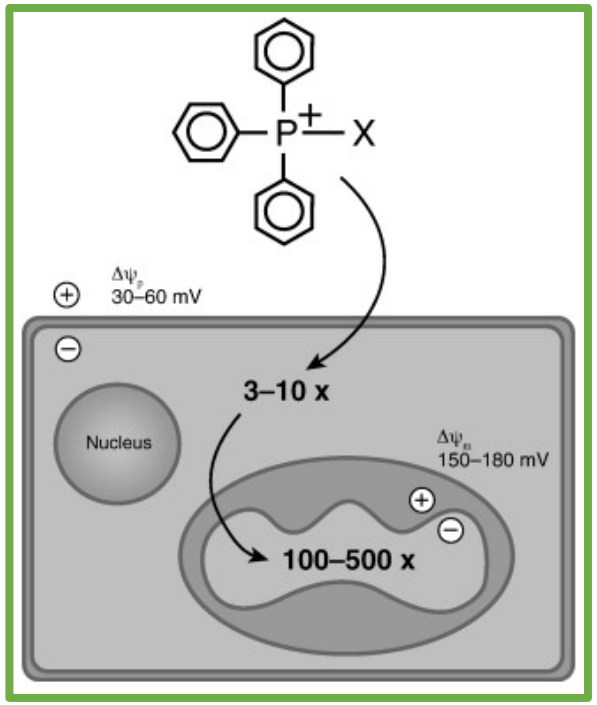
- cationic triphenylphosphonium azide for the mitochondrial probe
- tertiary amino azide for the lysosomal probe
- sulfonated azide for the plasma-membrane-specific probe
- perfluorinated azide for the ER probe



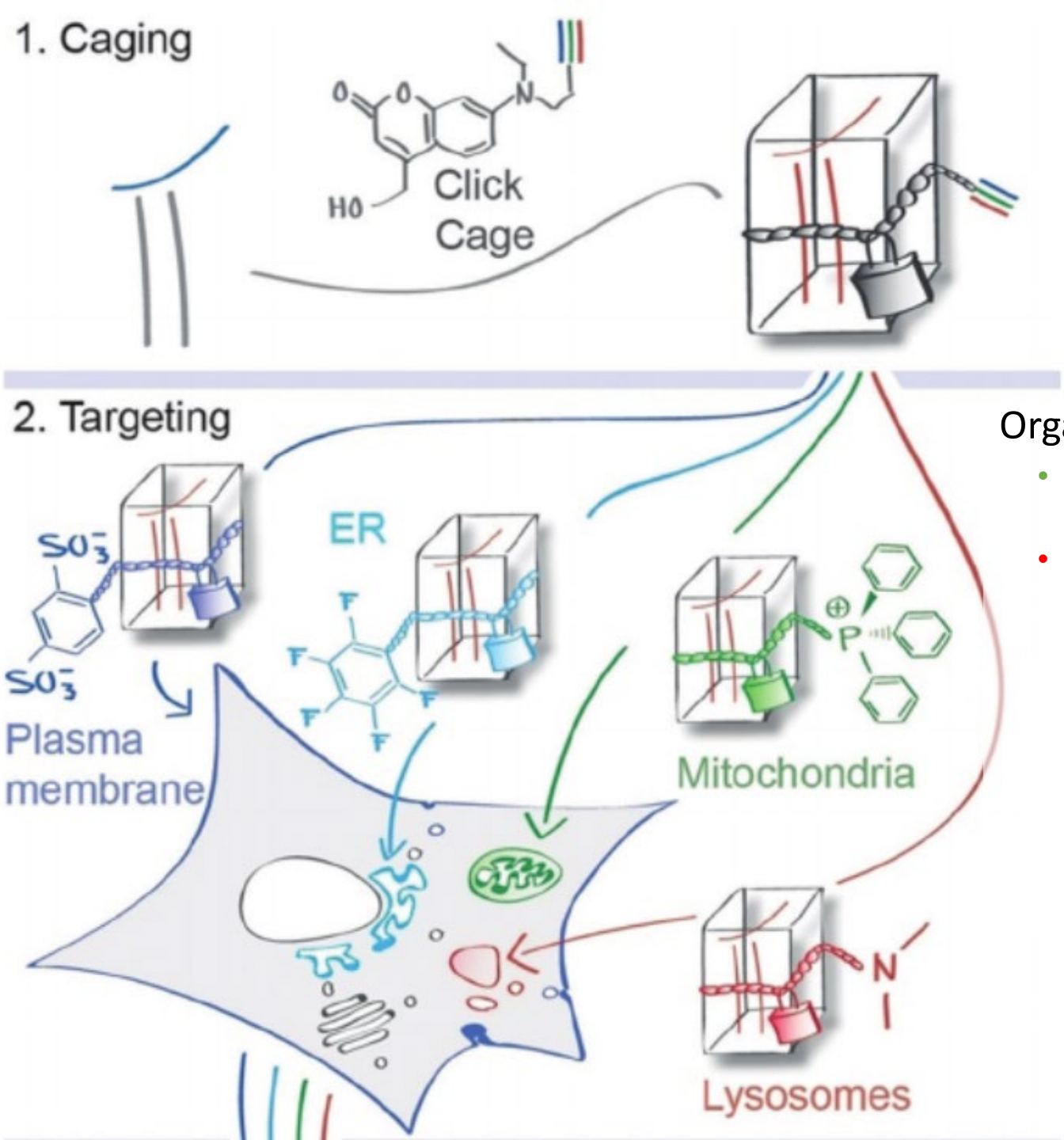


Organelle specific modifications via click chemistry

- cationic triphenylphosphonium azide for the mitochondrial probe
  → guidance via membrane potential



Murphy 2008



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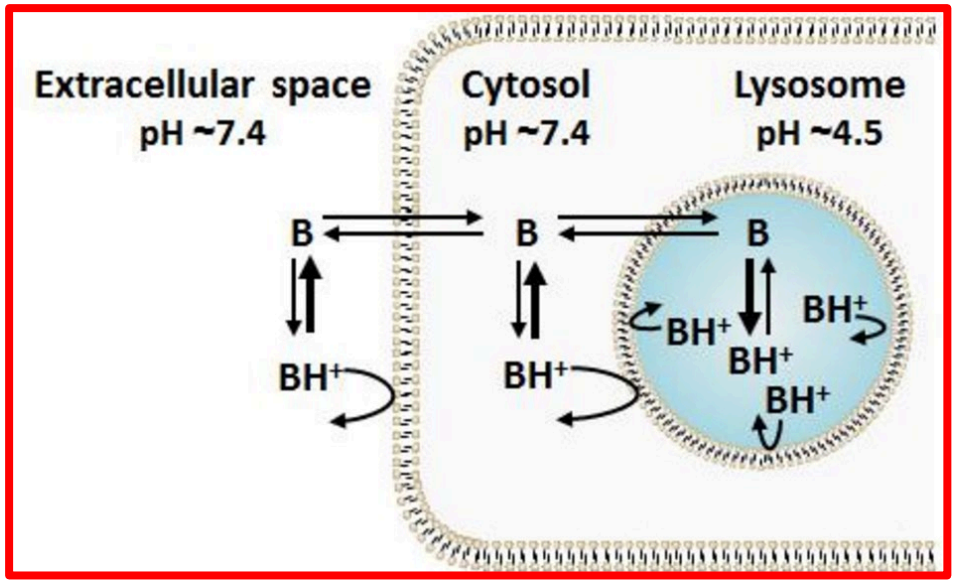
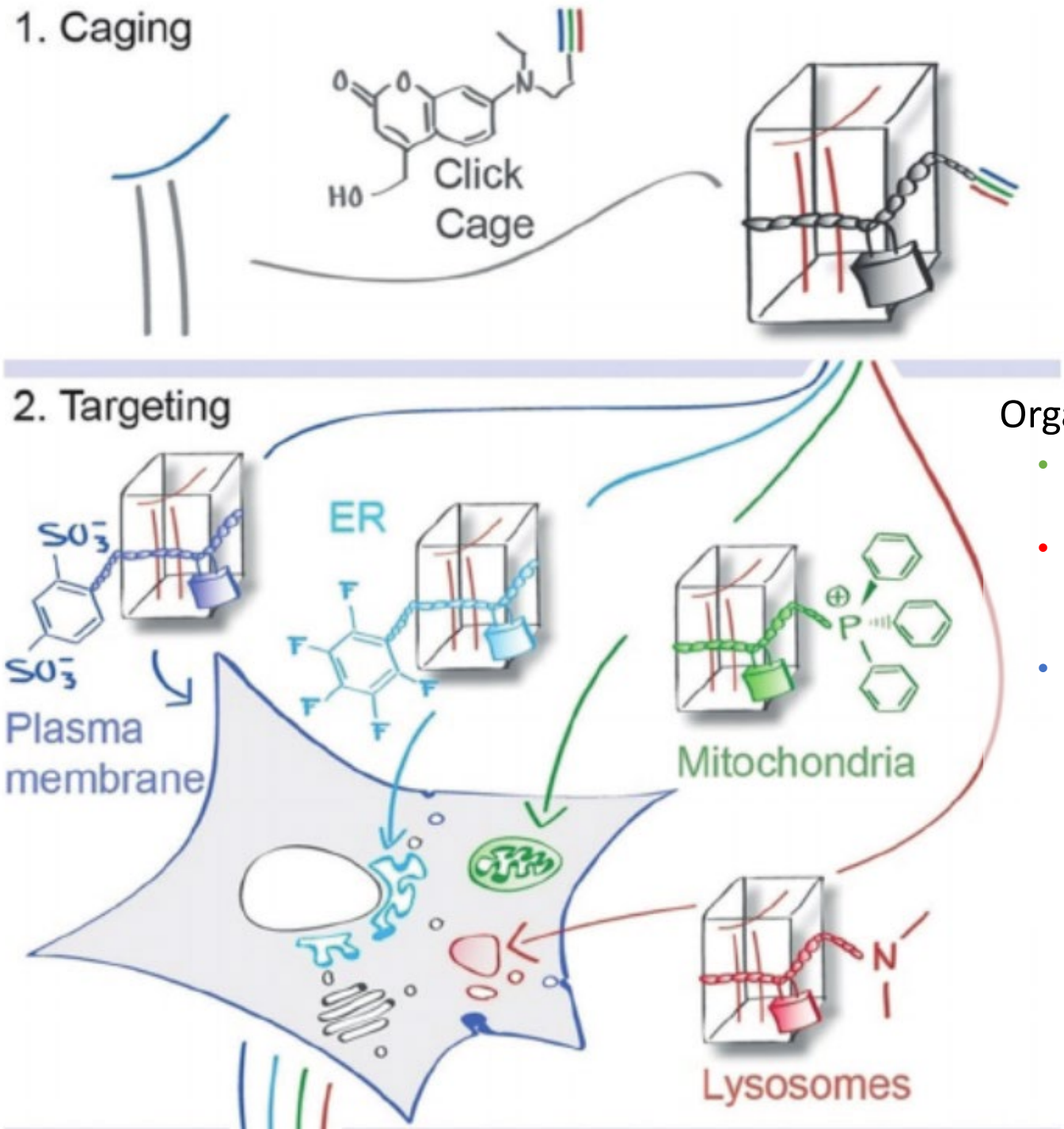
  → many amines are "lysosomotropic" due to low pH in lyso compared to cytoplasm, and depending on pKa of amine



Giraldo 2014



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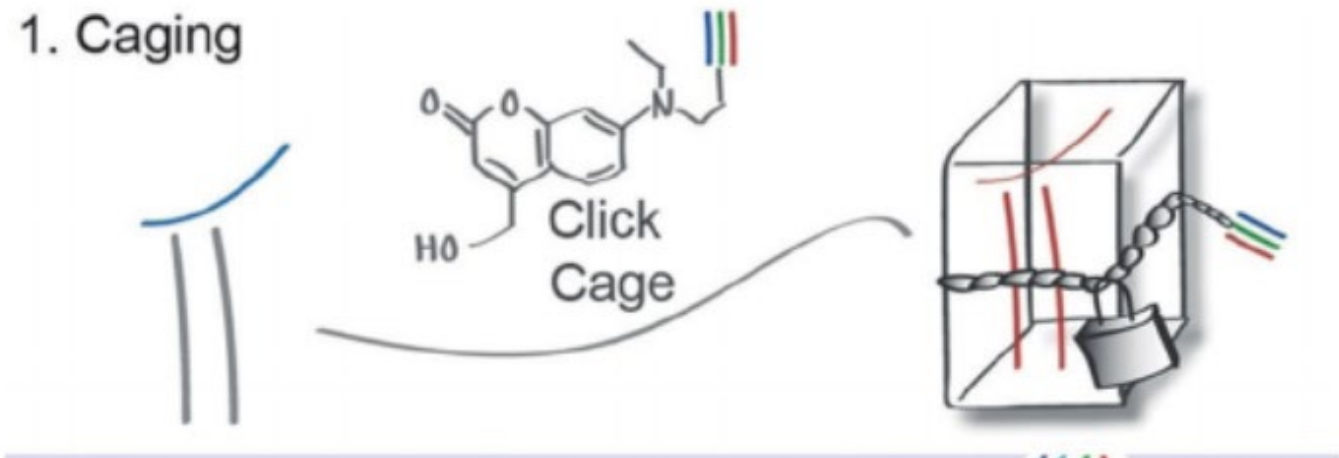
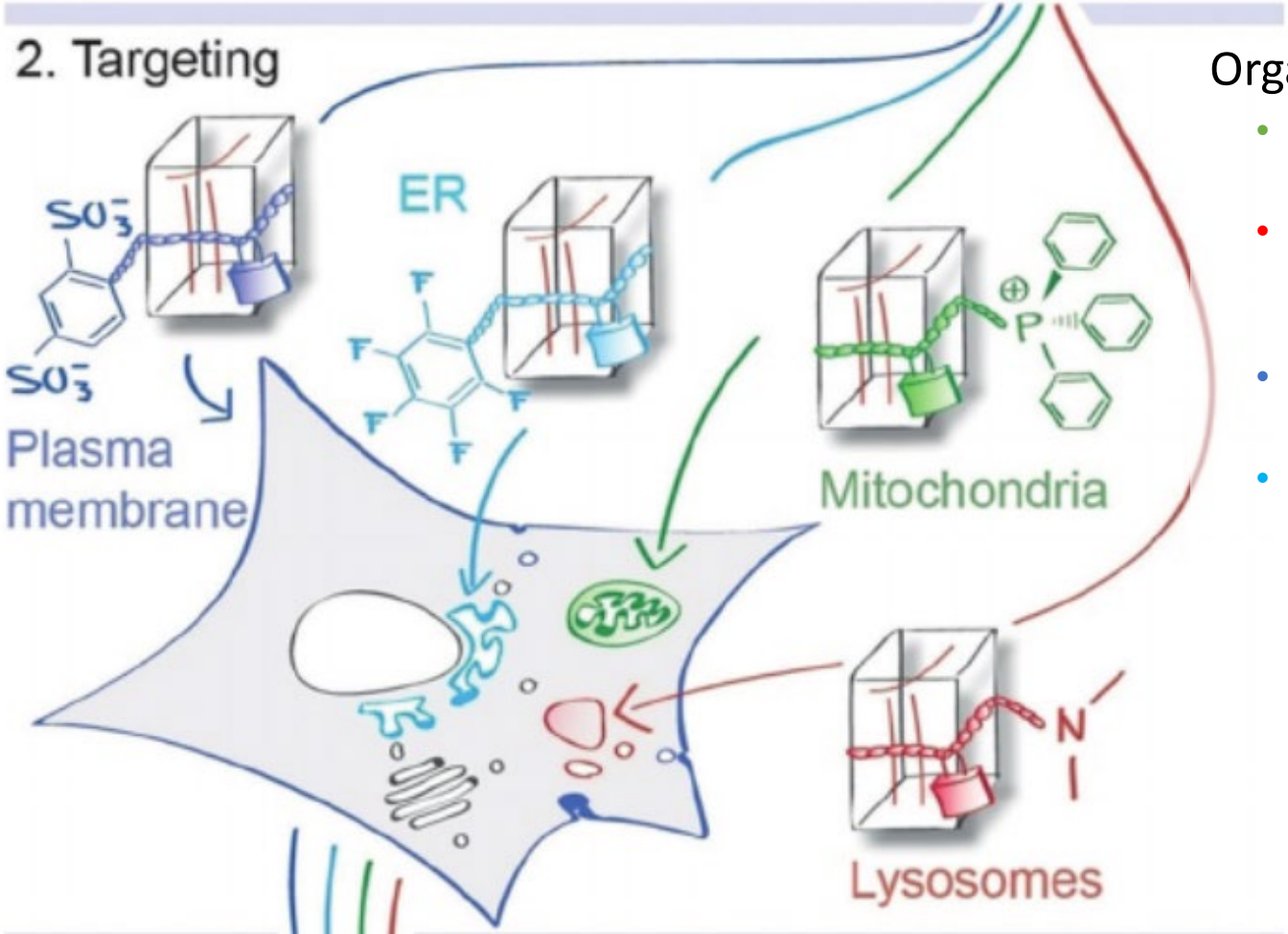
  → sulfate is a hydrophilic anion, cannot pass PM easily





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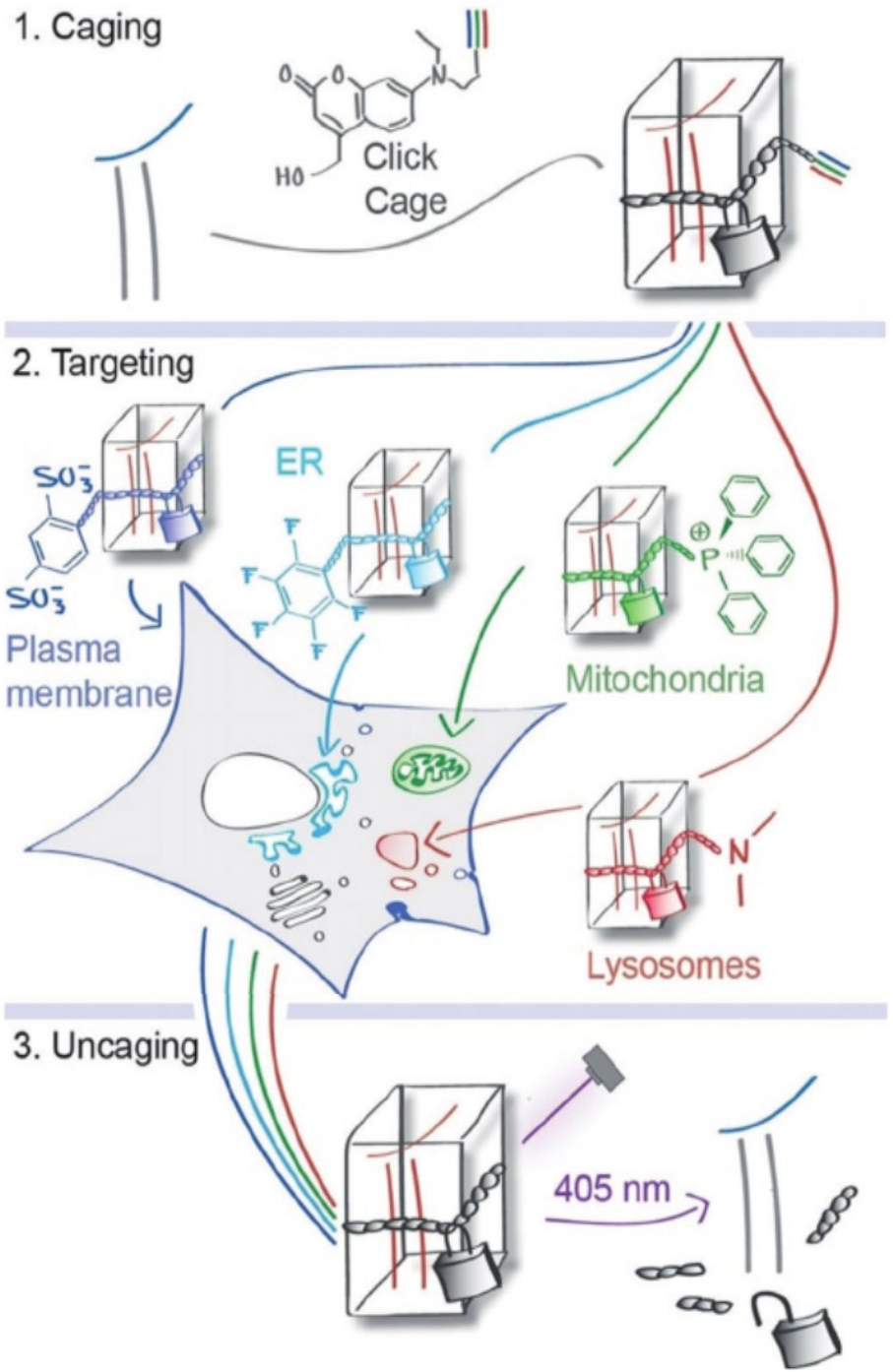
  → sulfate is a hydrophilic anion, cannot pass PM easily
- perfluorinated azide for the ER probe
  → hydrophobic and amphipathic properties confer a preference for the cholesterol-poor ER membranes

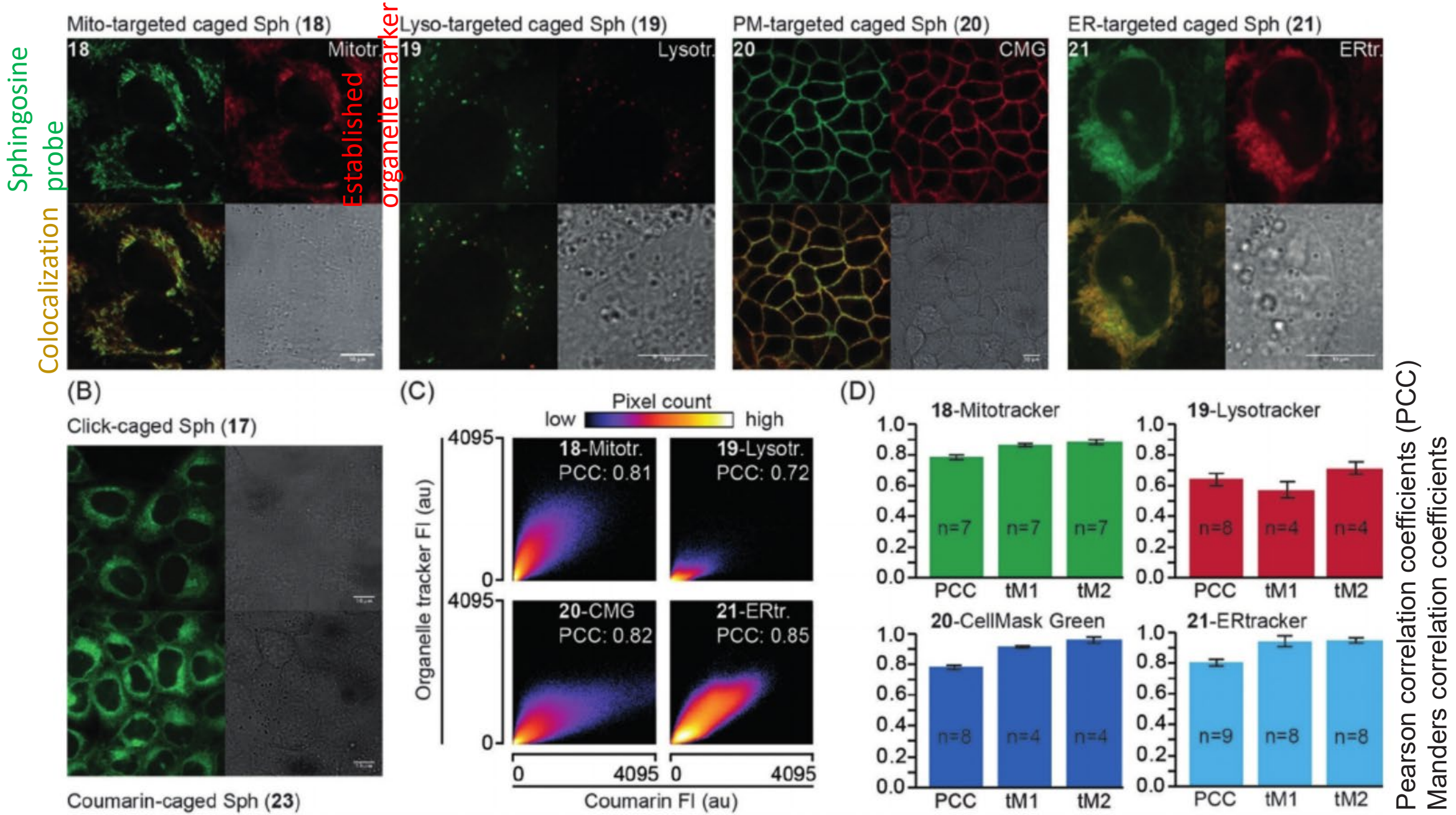


Depending on molecule chemistry, the photoactivatable lipid is integrated into the corresponding organelle

Uncaging works the same for all probes, via 405 nm laser illumination

→ Uncaged messenger molecule (arachidonic acid or sphingosine) can exert its function specifically in an organelle-specific membrane

Validation of organelle-specific localization



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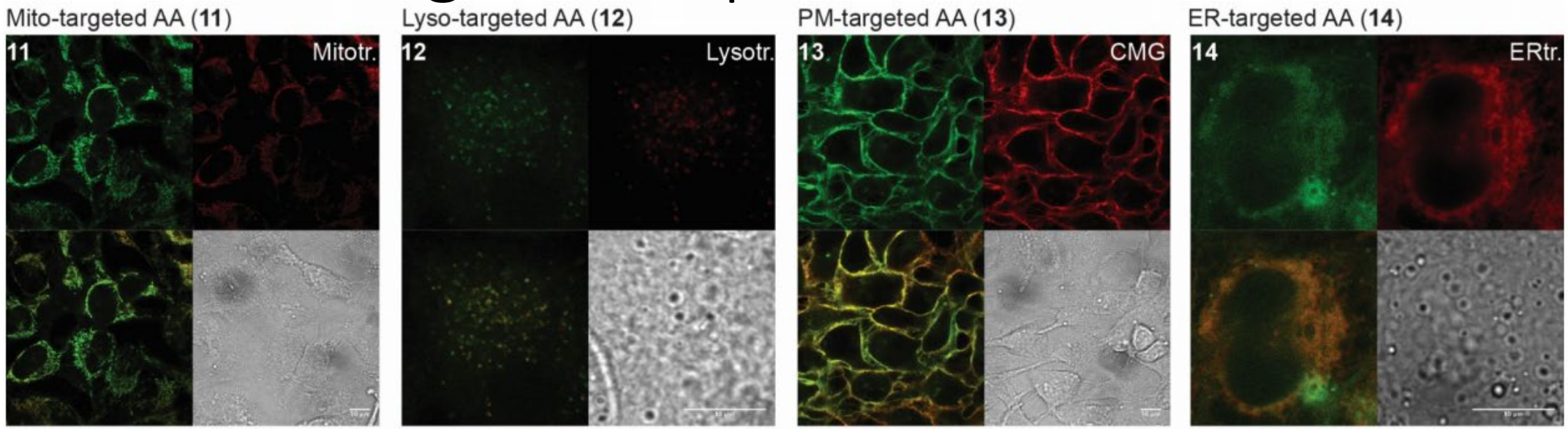
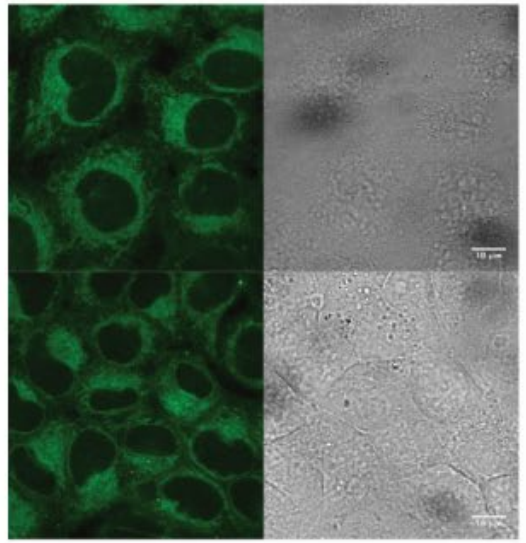


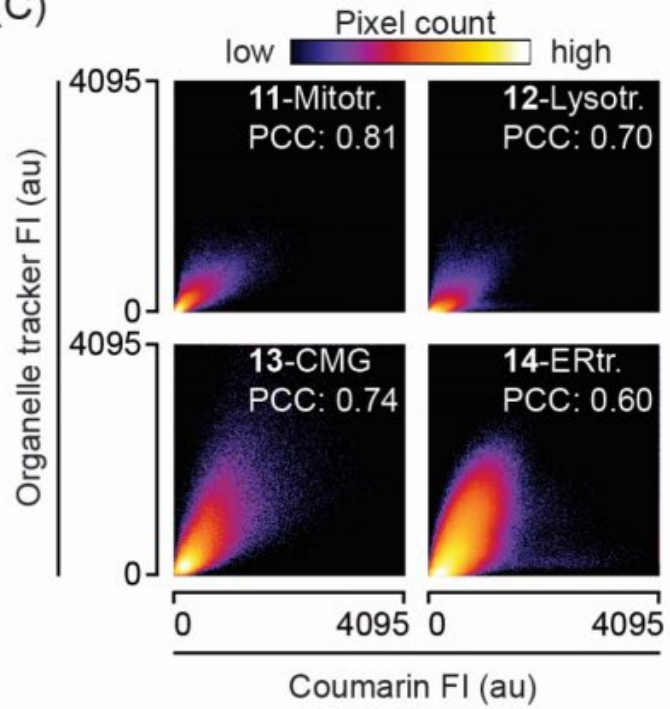
(B)

Click-caged AA (**6**)

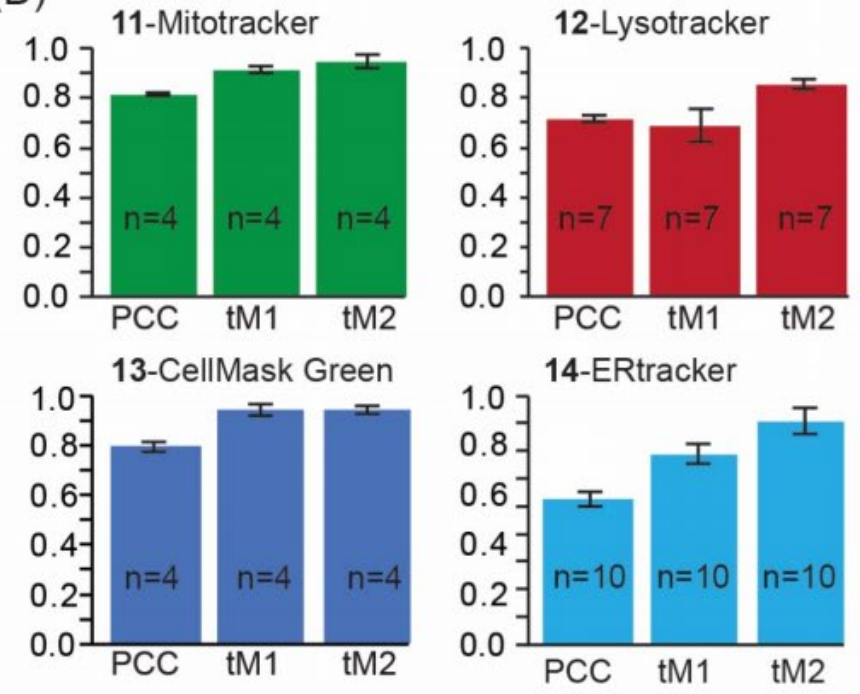


Coumarin-caged AA (**22**)

(C)

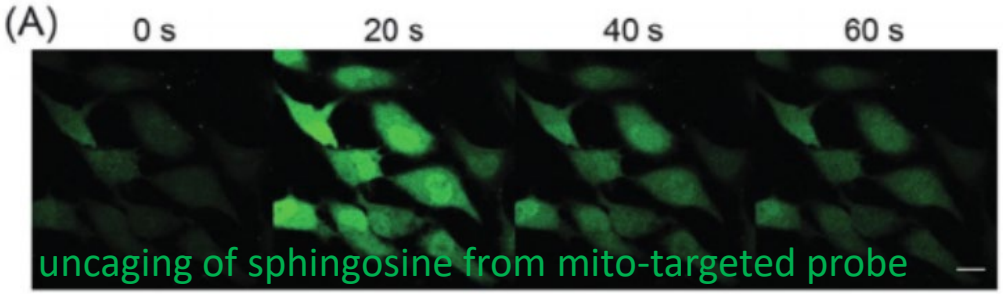


(D)



Uncaging of arachidonic acid and shingosine = messenger functionality?

- Measuring of Ca2+ currents:
  - HeLa cells were loaded with the calcium indicator Fluo-4-AM and the respective caged compounds
  - Uncaging with 385 nm LED



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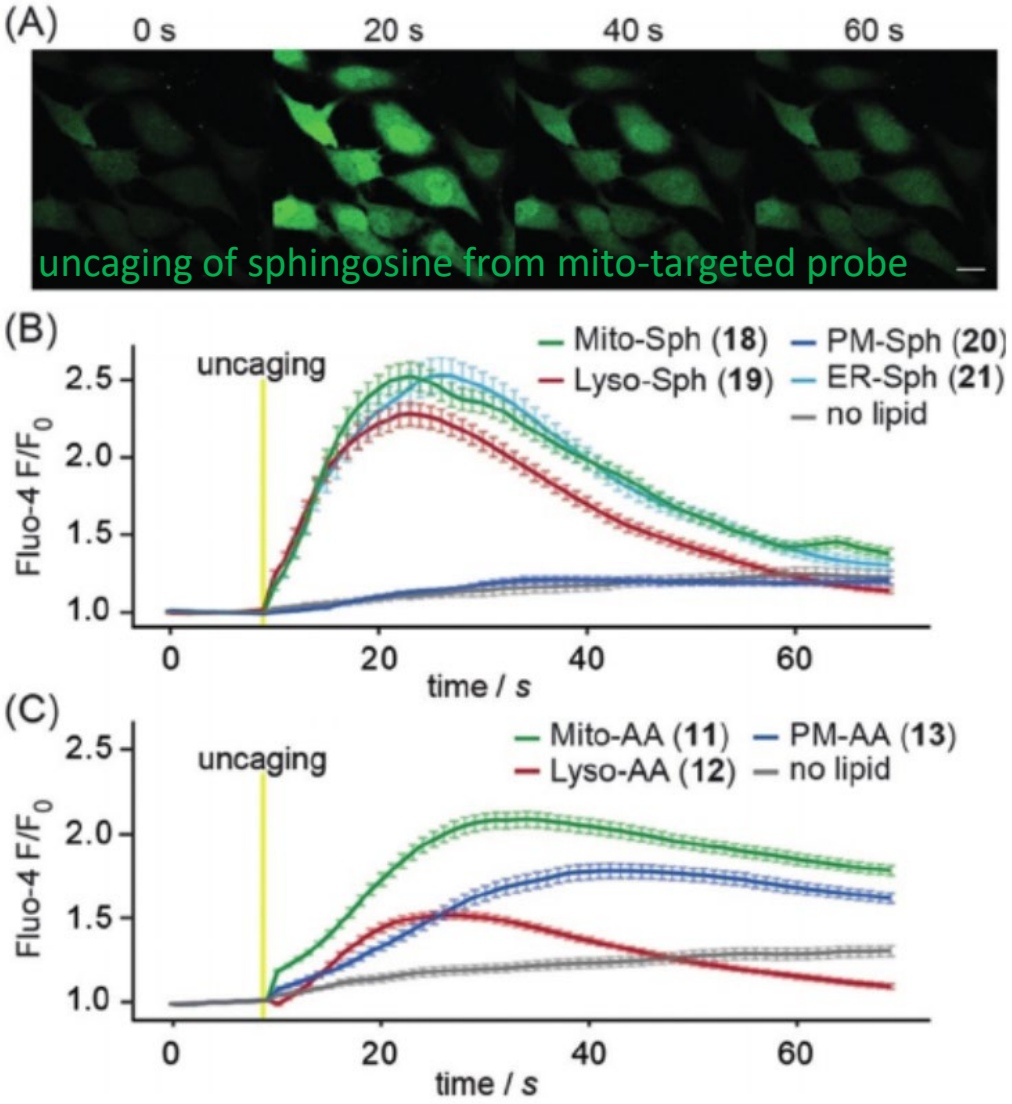
→Arachidonic acid-uncaging at Lyso elicted much less current than at PM and mito



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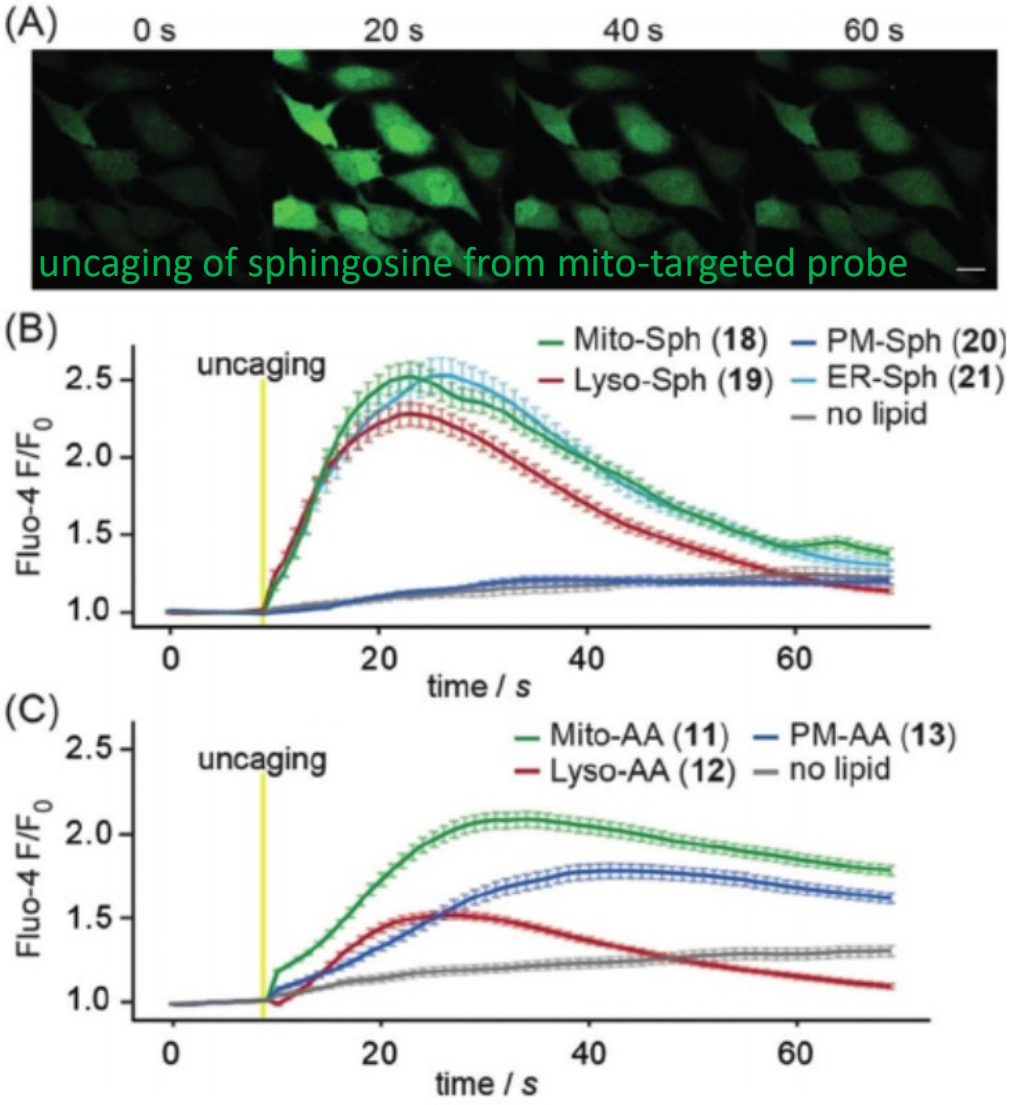
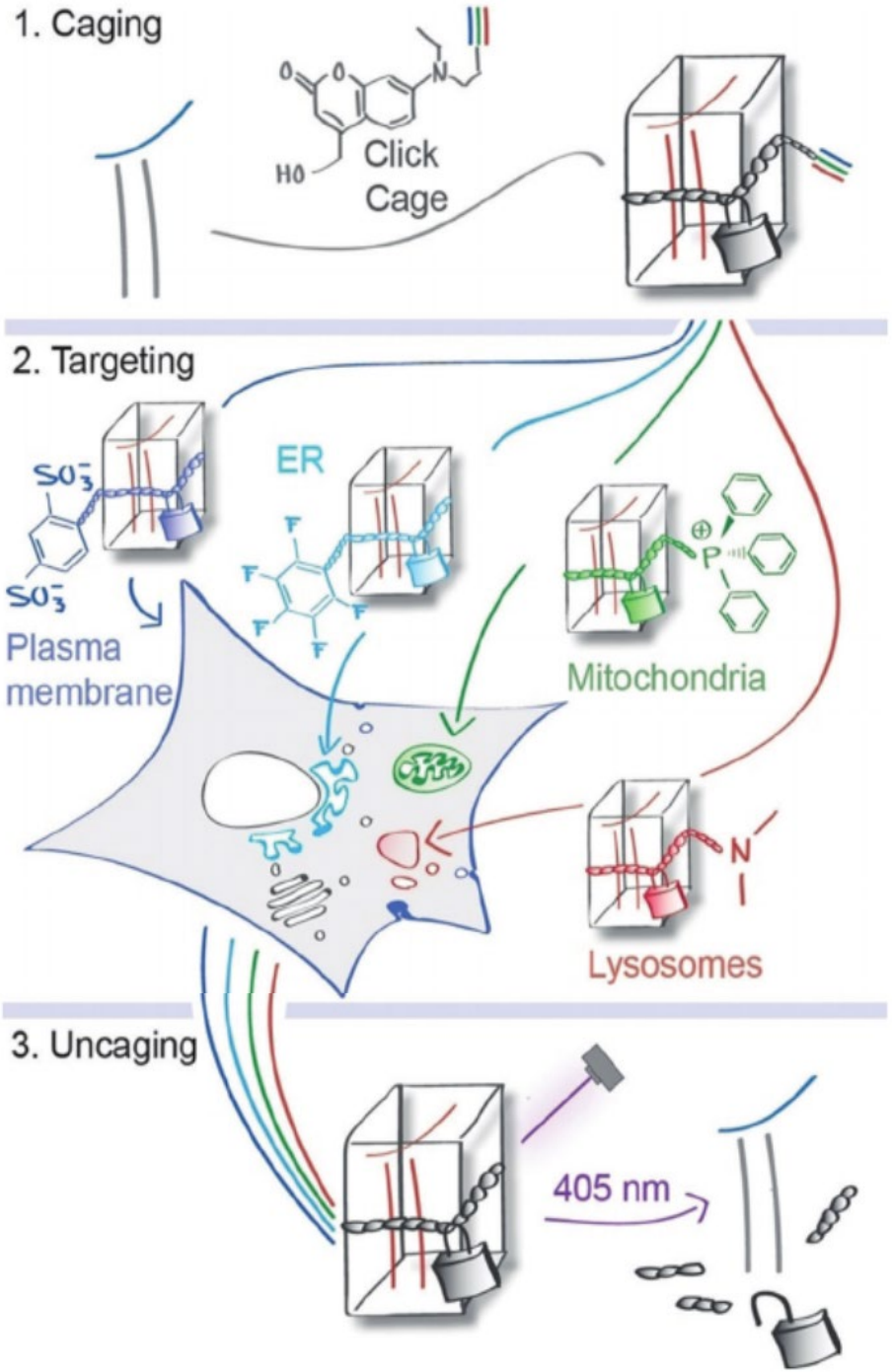
→in line with the localization of the main known intracellular targets of
  - →sphingosine: TPC1, in endosomes and lysosomes, and
  - →arachidonic acid: GPR40, at the plasma membrane

→Reason for transients after mito-targeted uncaging is unclear: either mito storage release or rapid transport of the messengers to respective sites of action



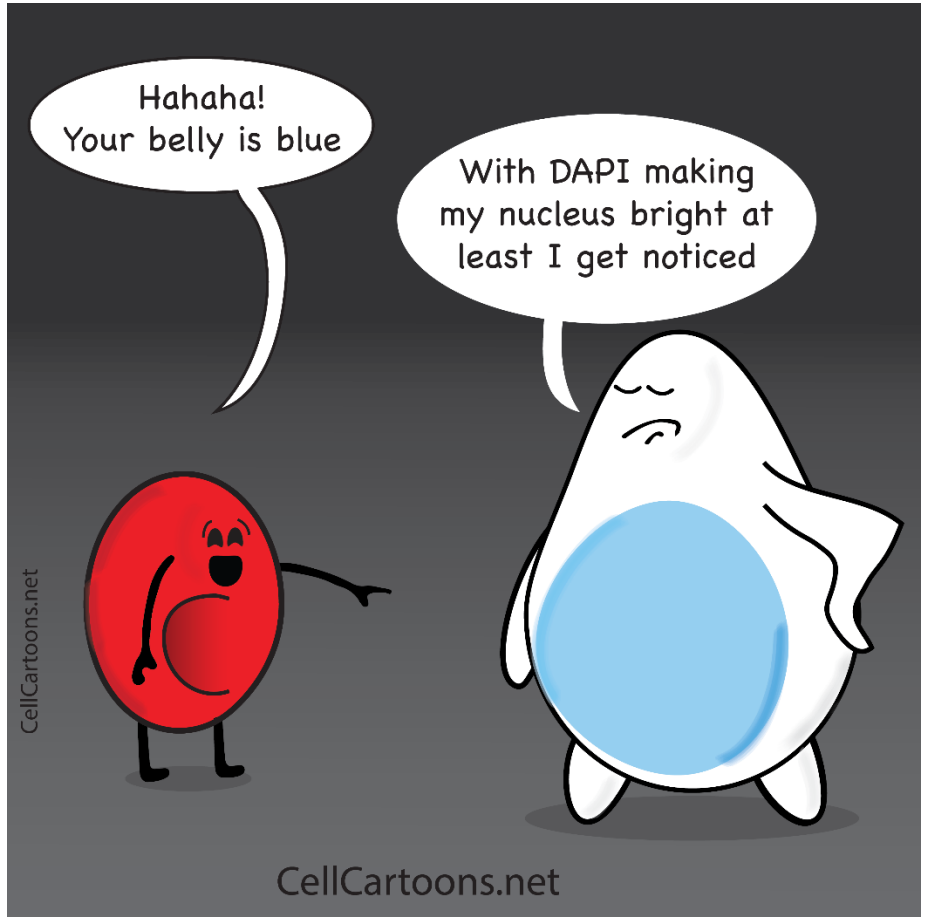


Summary

- Inducible photorelease of lipid messengers
- Temporal control: induction but not termination
- Spatial control: single cell / wide field illumination
- Subcellular spatial control: Organelle-targeting
- Potential for relatively simple generation of more probes targeting other organelles

Drawback for our interest: probes are localizing in organelles with the organelle-specific properties

- Lysosome-targeted probe may go to lyso due to pH, but if pH is altered (as in vacuolar blowing up) it might not stay there
- Only typical / intact organelles may be targeted, we cannot find out the origin of the vacuolar membrane with this


Hahaha!
Your belly is blue
With DAPI making my nucleus bright at least I get noticed
CellCartoons.net
CellCartoons.net